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PRINCIPAL INVESTIGATOR: Craig D. Shriver

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Bethesda, MD 20817

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Dr. Craig D. Shriver, MD FACS, COL, MC, USA – Principal Investigator
Ms. Lee Bronfman, RN, MA, CCRP – Administrative Director

email: craig.d.shriver.mil@mail.mil

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14. ABSTRACT

The WRNMMC Clinical Breast Care Project/Winder Research Institute will help lead the way in the 21st century in the crusade against breast disorders. The project will utilize a multidisciplinary approach as the standard of care for treating breast diseases and breast cancer. This multidisciplinary model integrates prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advances in risk reduction, informatics, tissue banking and research. These efforts focus on decreasing the morbidity and mortality of breast cancer among American women.

15. SUBJECT TERMS

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Breast Cancer Translational Research Center of Excellence FY12-14
Annual Report
COL Craig D. Shriver, M.D.; Principal Investigator and Director

I. INTRODUCTION

Objective/Hypothesis: Utilize and extend our unique DoD biorepository of well characterized biospecimens from a broad subset of patients with breast cancer and other breast diseases to broaden our knowledge of the etiology and pathology of breast disease specifically focused on breast cancers affecting the readiness of active duty women. Leverage the technological and information technology advances in genomic, proteomic, and total metabolomics research to further our understanding of breast cancer through discoveries in molecular biology, pathway analysis and systems biology that can be readily translated into the clinic.

Specific Aims
This project is structured around three major themes focused on breast cancer development in at-risk women of active duty military age: clinically relevant molecular profiles; evaluation of genetic and military-specific exposures risk; and tumor biology. These themes include research across the five BC- COE pillars: (1) Breast Cancer Risk Reduction; (2) Biorepository; (3) Focused Research; (4) Biomedical Informatics; and (5) Translational Clinical Care.

Study Design: The project utilizes a multidisciplinary approach for researching breast diseases and breast cancer focused on the military at-risk population in order to enhance Readiness of The Total Force. This multidisciplinary model integrates prevention, screening, diagnosis, treatment and continuing care, but the project is further unique in the incorporation of advances in risk reduction, biomedical informatics, tissue banking and translational research. The project is based on a Discovery Science paradigm, leveraging high-throughput molecular biology technology and our unique clinically and pathologically well-characterized tissue repository with advances in biomedical informatics leading to hypothesis-generating discoveries that are then tested in hypothesis-driven experiments.

Relevance: The BC-COE is the continuation of the Clinical Breast Care Project (CBCP) that has been ongoing for 14 years. Its uniqueness and relevance has been attested to by numerous outside world-class cancer experts, from the innumerable public scientific and invited lecture presentations made by CBCP PI and investigators over the years, as well as by the extensive peer-reviewed publication record of CBCP researchers. CBCP has developed the world's largest best-characterized and acquired biorepository of human breast tissues and biospecimens. CBCP has one of the few fully integrated genomic and proteomic molecular biology research programs in the nation devoted exclusively to research in breast diseases that is linked directly to the clinic and the patients (translational research) and which is focused on the problem of breast cancer as it relates to the military population.

Background

Breast cancer is the most common non skin-related malignancy among women in the western world. It accounts for one-third of all cancers diagnosed. Age is the single most important risk factor for the development of breast cancer, as incidence and mortality both increase with age. However, a significant number of breast cancers are diagnosed among young women and this shift towards younger women developing breast cancer has increased in the past five years. Each year, over 10,000 new breast cancer cases are detected in women under the age of 40. Over 90% of these occur among women aged 30-39 years and 8 women per 10,000 in this age group die from breast cancer every year. Breast cancer is the single leading cause of death in women aged 40-49 years. Despite the low absolute risk of breast cancer in women under 40 years of age, the incidence is increasing in this age group. The incidence in younger women is probably underestimated based on the current understanding of the biology of breast cancer. The focus of the Breast Cancer Translational Research Center of Excellence (BC-COE) is to work towards decreasing the morbidity and mortality of breast cancer among American women with a specific focus on the problem as it pertains to the active duty military population, an increasing number and proportion of which are female and are in this under-40 age group of increasing breast cancer development, risk, and poorer outcomes. As all jobs and positions in the military are now available to women including combat positions, the increasing incidence of breast cancer in younger (military-age) women and the increased lethality of that subtype of breast cancer, coupled with the military's critical reliance on a Total Force of all personnel inclusive of a high and increasing percentage female, demands a continued effort of the DoD through the BC-COE to focus on surveillance, screening, early detection, curative treatments, and post-treatment Return To Duty Survivorship programs. The BC-COE has had a 14 year history of doing just that, and we are robustly moving into the future by targeting our valuable resources to the active duty military cancer problem, aligning ourselves with other DoD and federal agencies in order to increase efficiencies and allow best use of government funds, and ensuring we are in complete alignment with the DoD QUAD AIM with the central pillar of our efforts focused on READINESS of the Total Force.

Hypothesis/Rationale/Purpose

The Walter Reed National Military Medical Center and Windber Research Institute have partnered in the Clinical Breast Care Project (CBCP) since the inception of the project (April 2000). This program has become a leader in the national fight against breast disorders and cancer. Recognition of this leadership has prompted Congress to establish the CBCP as the Breast Cancer Translational Research Center of Excellence (BC-COE), one of five centers so designated by Congress in 2010. The project utilizes a multidisciplinary bed-to- bench- to-bedside (translational medicine) approach as the standard for treating and studying breast diseases and breast cancer. This multidisciplinary model integrates advances in risk reduction, prevention, screening, diagnosis, treatment and continuing care with cutting edge research incorporating advanced methods from biomedical informatics, tissue banking, high throughput biology and translational research. These efforts focus on decreasing the morbidity and mortality of breast cancer among American women and specifically active duty military women and DoD beneficiaries.

The BC-COE currently utilizes the facilities, resources and expertise of the Walter Reed National Military Medical Center (WRNMMC), the Windber Research Institute (WRI), the Windber Medical Center (WMC) and the Anne Arundel Medical Center (AAMC). The BC-COE fosters a collaborative and collegial working relationship with its partners, other government agencies to include the National Cancer Institute (NCI), academic institutions, commercial/industry leaders, and other non- profit organizations. The CBCP has a five pronged approach to the study and treatment of breast disease based on five interlocking pillars: ***(1) Breast Cancer Risk Reduction; (2) Biorepository; (3) Focused Research (including: Genomics, Proteomics, and Metabolomics Research); (4) Biomedical Informatics; and (5) Translational Clinical Care.***

The overall purpose of the BC-COE is to provide a balanced environment between the two competing and yet complementary research paradigms of hypothesis-driven research and hypothesis-generating research, in a translational research organization that unites clinical capabilities (patients, nurses, clinicians) with research capabilities (genomics, proteomics, metabolomics, immunohistochemistry and whole genome DNA sequencing) to analyze molecular and developmental pathways that are central to the diagnosis and treatment of breast disease. The critical foundations to this approach are provided by the world-renowned CBCP tissue biorepository and biomedical informatics platforms.

There are three broad areas where the BC-COE stands poised to make continued major contributions to breast cancer research and its translation into clinical practice. These areas include the identification of molecular profiles of disease with high clinical relevance, deepening our understanding of the genetic and demographic risks of breast disease, and the enhancement of our understanding of breast tumor biology. These three themes are supported by the five pillars of the BC-COE. There is no doubt that our understanding of the biology of Breast Cancer in all of its various forms and manifestations remains incomplete, despite great advances in recent years by our group and others. We know based on outside agencies' assessment of our high-value repository of biospecimens, our strong biomedical informatics infrastructure, and our clinical-translational research base with strong internal and external collaborations puts us in an excellent position to make continued unique and unparalleled contributions to the understanding of breast diseases and cancer that will have impact on the quality of life for breast cancer patients and their families, specifically and uniquely focused on active duty military, beneficiaries, and contributing to overall military readiness.

Clinically Relevant Molecular Profiling: This is a cross-cutting theme with clinical, risk assessment and basic research components. The primary focus of this theme is to evaluate the utility of existing molecular profiles that have relevance to risk assessment, diagnosis, prognosis and therapy in a clinical setting and to discover new profiles that can be evaluated in the clinic. Projects within this theme have well defined translational goals. The further development (as this is a multi-year ongoing project within CBCP already) of comprehensive and highly informative molecular profiles will be a foundation for the continued and more specific development and delivery of personalized/individualized medicine. A variety of research modalities will be used to identify these profiles including immunohistochemistry, gene and protein expression analysis and genetic profiling including Next Generation DNA Sequencing. Two major new initiatives are

outlined below: one involving the development and testing of clinically relevant immunohistochemical profiles for disease stratification and therapeutic guidance; and the other using complete genomics sequencing of tumor and matched normal DNA to develop clinically relevant profiles that will predictably aid in disease diagnosis, prognosis and therapy selection.

Genetic Risk: The rapid developments of high throughput genotyping and genomic sequencing of individuals has reminded the research community of the power of family studies in the assessment of genetic risk. Evaluating family risk and translating that into individual risk is the primary goal of this theme. There is both clear clinical relevance and a strong basic research component to this theme. Understanding the underlying biology of observed racial disparities in disease prevalence, presentation and outcome will also be a major part of this effort. The interaction of the theme with the Risk Reduction pillar of the BC-COE and the number of projects outlined below that deal with research into the basis of the observed racial disparities in breast cancer morbidity and mortality point out the relevance of this theme to the overall goals of the BC-COE and its historic and ongoing role as the DoD's breast cancer research project.

Tumor Biology: A unique combination of resources and expertise put the BC-COE in a strong position to further our understanding of the basic biology of breast disease including breast cancer. Many of the projects outlined in the Focused Research pillar address basic problems associated with tumor heterogeneity. The tumor microenvironment and stromal interactions, metastasis and recurrence, as well as the role of cancer stem cells and tumor evolution affecting the efficacy of treatment are emphasized. We and national science groups firmly believe that a robust understanding of breast tumor biology is a key to the successful translation of the research preformed at the BC-COE to the clinic.

II. BODY

Goals during this Annual Period:

- Decrease morbidity and mortality of breast cancer amongst American women with particular focus on the active duty female population demographic characteristics and risks. The BC-COE building upon the five pillars of the CBCP will continue to help lead the fight against breast disorders and cancer.

- Continue to further develop and refine our comprehensive breast care center/system with a multidisciplinary team approach that enables health care providers to work towards the common goal of reducing the morbidity and mortality caused by breast disease in the most cost-effective way possible.

- Empower women afflicted with breast cancer and other breast disorders, with the decision-making tools and an environment that enhances their quality of life and meets psychosocial needs of the patients and their families, as well as returning active duty members to duty as quickly as possible.

- To continue, support, and grow our existing world-class biorepository of biospecimens that enable research into diseases of the breast.

- Support our existing research facilities that drive world-class high-throughput translational research in a cost-effective manner.

- Refine our integrated computational and biomedical informatics infrastructure with an integrated data warehouse that forms the foundation for analysis of research findings leading to new and actionable knowledge related to diseases of the breast.

- Empower the clinical staff with a physician decision support system incorporating our evolving understanding of breast cancer and other breast diseases from research both within BC-COE and outside.

Pillar Specific Goals and Objectives:

I. Breast Cancer Risk Reduction:

<u>Objectives</u>:

a. To collect data on all female patients 18 and older who present to the CBCP Breast Center of Excellence at Murtha Cancer Center at Walter Reed National Military Medical Center - Bethesda and are found to be at an increased or elevated risk for developing breast cancer.

b. To utilize this database to analyze the diagnosis, treatment, and treatment outcomes for patients found to be at an increased risk for developing breast cancer. Analysis includes but is not limited to: risk factors for developing breast cancer, effectiveness of various modalities of risk-reduction treatment (medical, surgical), and actual risk of developing cancer.

<u>Background:</u>

Each year, approximately 200,000 women in the United States are diagnosed with breast cancer, and one in nine American women will develop breast cancer in her lifetime. But hereditary breast cancer — caused by a mutant gene passed from parents to their children is rare. Estimates of the incidence of hereditary breast cancer range from between 5 to 10 percent to as many as 27 percent of all breast cancers.

In 1994, the first gene associated with breast cancer — BRCA1 (for BReast CAncer1) was identified on chromosome 17. A year later, a second gene associated with breast cancer — BRCA2 — was discovered on chromosome 13. When individuals carry a mutated form of either BRCA1 or BRCA2, they have an increased risk of developing breast or ovarian cancer at some point in their lives. Children of parents with a BRCA1 or BRCA2 mutation have a 50 percent chance of inheriting the gene mutation.

In 1995 and 1996, studies of DNA samples revealed that Ashkenazi (Eastern European) Jews are 10 times more likely to have mutations in BRCA1 and BRCA 2 genes than the general population. Approximately 2.65 percent of the Ashkenazi Jewish population has a mutation in these genes, while only 0.2 percent of the general population carry these mutations.

Further research showed that three specific mutations in these genes accounted for 90 percent of the BRCA1 and BRCA2 variants within this ethnic group. This contrasts with hundreds of unique mutations of these two genes within the general population. However, despite the relatively high prevalence of these genetic mutations in Ashkenazi Jews, only seven percent of breast cancers in Ashkenazi women are caused by alterations in BRCA1 and BRCA2.

Medical Application:

Not all hereditary breast cancers are caused by BRCA1 and BRCA2. In fact, researchers now believe that at least half of hereditary breast cancers are not linked to these genes. Scientists also now think that these remaining cases of hereditary breast cancer are not caused by another single, unidentified gene, but rather by many genes, each accounting for a small fraction of breast cancers.

Hereditary breast cancer is suspected when there is a strong family history of breast cancer: occurrences of the disease in at least three first or second-degree relatives (sisters, mothers, aunts). Currently the only tests available are DNA tests to determine whether an individual in such a high-risk family has a genetic mutation in the BRCA1 or BRCA2 genes.

When someone with a family history of breast cancer has been tested and found to have an altered BRCA1 or BRCA2 gene, the family is said to have a "known mutation." Positive test results only provide information about the risk of developing breast cancer. The test cannot tell a person whether or when cancer might develop. Many, but not all, women and some men who inherit an altered gene will develop breast cancer. Both men and women, who inherit an altered gene, whether or not they develop cancer themselves, can pass the alteration on to their sons and daughters.

But even if the test is negative, the individual may still have a predisposition to hereditary breast cancer. Currently available techniques can't identify all cancer-predisposing mutations in the BRCA1 and BRCA2 genes. Or, an individual may have inherited a mutation caused by other genes. And, because most cases of breast cancer are not hereditary, individuals may develop breast cancer whether or not a genetic mutation is present.

Genetic counselors can help individuals and families make decisions regarding testing. For those who do test positive for the BRCA1 or BRCA2 gene, surveillance (mammography and clinical breast exams) can help detect the disease at an early stage. A woman who tests positive can also consider taking the drug tamoxifen, which has been found to reduce the risk of developing breast cancer by almost 50 percent in women at high risk. Clinical trials are now under way to determine whether another drug, raloxifene, is also effective in preventing breast cancer.

The field of oncology/surgical oncology is an ever- changing one with new developments in both diagnosis and treatment. We propose to collect data from all patients in the WRNMMC Comprehensive Breast Center determined to be at an elevated risk for developing breast cancer in order to assess risk factors in this population for developing the disease and track outcomes of preventive and therapeutic interventions. Analysis of outcome will include comparison of various treatment modalities/regimens with regard to efficacy, risks for failure, complications, and overall morbidity/mortality/survival. The patient population of WRNMMC can provide a significant number of patients to compare/contrast our findings with those of our civilian counterparts, specifically the Joyce Murtha Breast Care Center. The database will also allow us to analyze breast cancer risk data to provide scientific-based evidence that will guide the general surgeon and medical oncologist in optimal care of the patient at an elevated risk for developing breast cancer.

Plan:

The Risk Reduction Clinic at WRNMMC is a multi-disciplinary program designed to identify, counsel and manage women at high risk for breast cancer. Patients receive an in-depth personal and family health history by a world renowned medical oncologist. A total of 385 were managed; 317 patients were seen and 68 telephone consults were conducted at Walter Reed National Military Medical Center.

Current research shows there are risk factors that may influence the development of breast cancer. Identifying people with these risk factors and implementing closer surveillance and risk reduction techniques may detect cancer earlier. Earlier detection of breast cancer leads to better prognosis and outcomes. Calculations of risk are based on computer models extensively validated as accurate in identifying women at high risk.

Subjects: All female patients age 18 and older, seen in the WRNMMC Comprehensive Breast Center at a high risk for developing breast cancer. Patients will have consented to WU # 01-20006, Tissue and Blood Library Establishment for Molecular, Biochemical, and Histologic Study of Breast Disease or WU # 01-20007, Creation of Blood Library for the Analysis of Blood for Molecular Changes Associated with Breast Disease and Breast Cancer Development. The core questionnaire completed by the patients contains the NCI Breast Cancer Risk Assessment Tool used to determine a patient's risk factor.

Study Design and Methodology: Patients being seen in the Comprehensive Breast Center at WRNMMC or at the JMBCC in Windber, PA will be assessed for their risk of developing breast cancer by their history of LCIS or ADH or by applying the NCI Breast Cancer Risk Assessment Tool. Identified high-risk patients will be referred to the CBCP Risk Reduction Clinic. Patients are confirmed to meet the inclusion criteria and consented to one of two core protocols. Information collected will include the data contained on the enclosed database forms and may include data from previous clinic visits. All applicable patients will be followed indefinitely according to the applicable protocol.

If patients are referred for genetic testing, as per the American Society of Clinical Oncology, counseling involves the following eleven points:

1. Information on the specific test being performed
2. Implications of positive and negative results
3. Options for estimation without genetic testing
4. Risk of passing a mutation to a child
5. Technical accuracy of the test
6. Possibility that the test will not be informative
7. Fees involved in testing
8. Risk of psychological distress
9. Risk of insurance or employer discrimination
10. Issues
11. Options for medical surveillance and screening following testing

It has been observed that healthy female relatives of individuals with ovarian or breast cancer tend to exaggerate their risk of incurring either form of cancer and, thus, accurate risk assessment is essential to quality genetic counseling for breast cancer. Breast cancer genetic counseling serves the goal of helping women to analyze their own and their relatives' risk of developing breast cancer.

In BRCA screening, genetic counselors offer services in compiling family histories, personalizing their individual risk profiles and, more recently, conducting genetic testing to empower healthy women of families stricken by breast cancer to alter their lifestyles and healthcare to ensure avoidance or early detection of breast cancer. In addition, breast cancer victims and healthy members of a single family can enable accurate screening of female family members by obtaining a sequence of their BRCA genes. Detection of a familial BRCA mutation in individuals outside of the Ashkenazi Jewish population requires time- consuming genetic analysis of a large number of affected and unaffected family members in order to identify the specific BRCA mutation for a particular family.

Testing positive or negative for a BRCA mutation is simply a risk assessment, not a certainty of experiencing or avoiding, respectively, breast cancer. Individuals with a BRCA mutation have an 80% risk of developing breast cancer by age 80. Therefore, 20% of BRCA mutation carriers never develop breast cancer. A first-degree relative of a carrier who tests negative for the mutation has the same breast cancer risk as women of the general population, namely 11%.

II. Biorepository:

- Continue to collect and store a broad spectrum of biospecimens from every patient undergoing a breast biopsy and/or breast surgery at WRNMMC, WMC, AAMC, and our affiliated hospitals that consent to participate in BC-COE IRB-approved protocols.

- Continue to collect and store biospecimens (blood) from women who are free of breast disease who consent to participate in BC-COE IRB-approved protocols to act as controls.

- Utilize the power of this extensive biorepository as a major resource for breast disease research.
- Leverage the BC-COE biorepository to maximize the utilization of the repository, with BC-COE leadership approval, for the overall benefit of breast cancer patients and research, as able and appropriate.

- Participate in national/international projects that can benefit from resources of the BC-COE biorepository.

Although there have been remarkable improvements in breast cancer diagnosis and management, most of the complex molecular mechanisms associated with the onset, progression and/or severity of breast cancer are still not well understood. As part of the BC-COE we carry out molecular, biochemical and histological analysis of breast tissue and/or blood and blood components from breast cancer patients to provide insights into the molecular mechanisms that may be relevant in the development of breast cancer and breast diseases. To achieve this aim, a large supply and a wide variety of good quality tissue samples are needed. Unfortunately, good quality donor breast tissue is extremely scarce and when available is often not backed by a comprehensive medical history and/or is not a good representation of the target population or study area. The non-availability of a steady and consistent supply of good quality tissue limits the systematic analysis of tissues and negatively impacts the generation of biologically useful information in research laboratories and by extension negatively impacts new findings that benefit clinical practice. The objective of this project is therefore the acquisition and banking of breast tissue, lymph nodes, serum/plasma and other blood derivatives from informed and consenting donors.

Since the inceptions of the Clinical Breast Care Project the Biorepository Pillar has been critical to the success of the project. As we move forward into the establishment of the BC-COE it is important to look at the success of the biorepository and to understand the firm foundation that it has laid for building the Center of Excellence.

The charts below show the cumulative patient accrual into the CBCP protocols and total number of specimens stored in our biorepository since 2002. These patients, who have been recruited and consented into the CBCP protocols at WRAMC, WRNMMC, AAMC, JMBCC and other participating CBCP clinical intake sites are the foundations of the translational research that has occurred within the CBCP and which will continue in the BC-TRCOE. From these patients we have collected and stored in our biorepository over 58,012 biospecimens (**Figure BB-1**) donated by 6,645 fully consented subjects to our IRB approved tissue and blood protocols. (**Figure BB-2**)

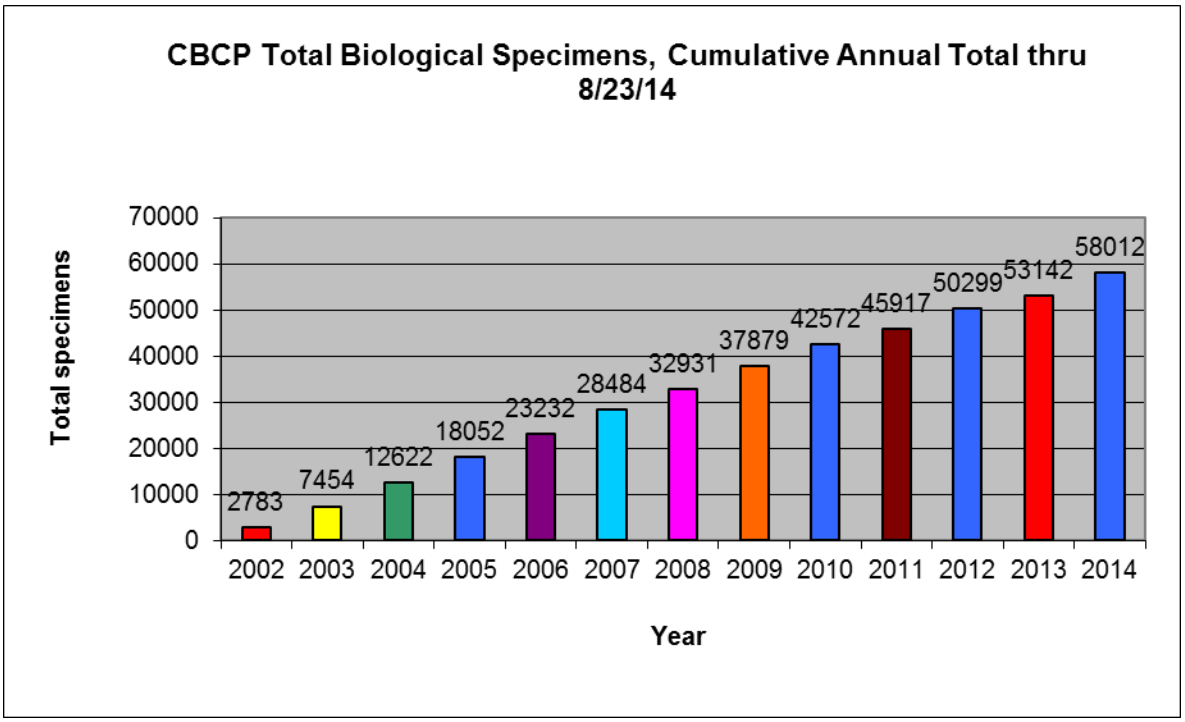


Figure BB-1 Total biospecimens collected and banked by the biorepository.

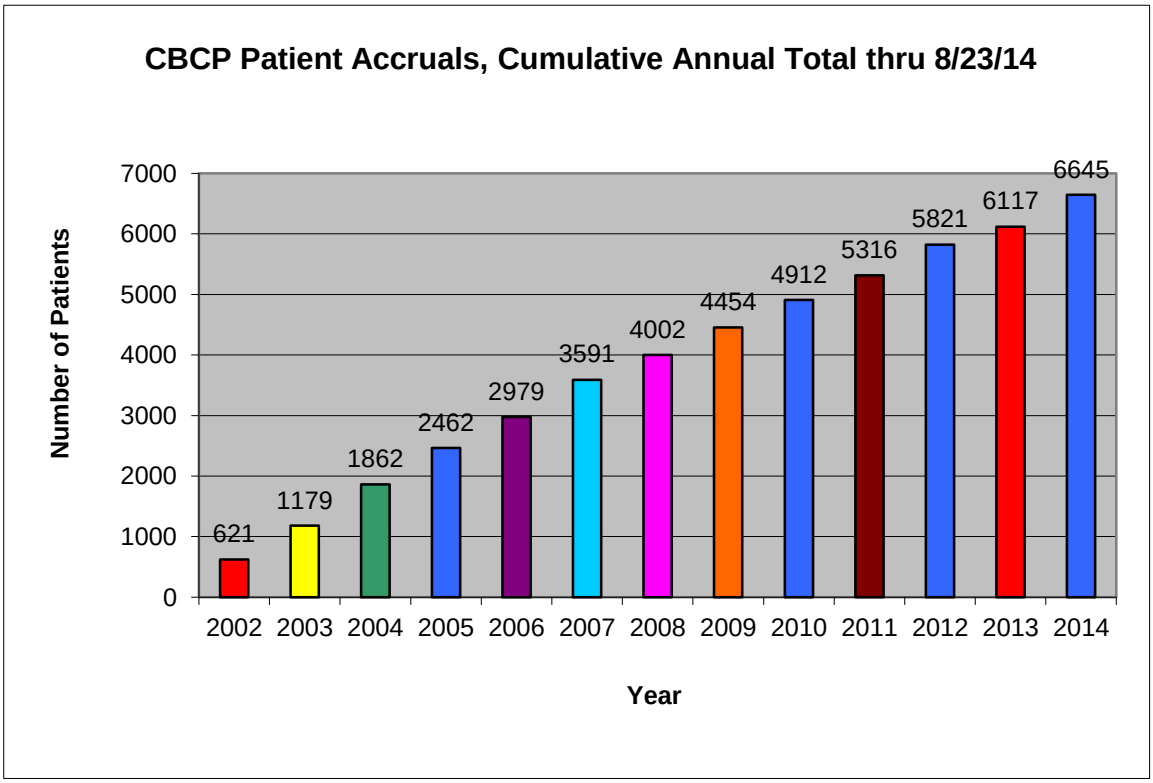


Figure BB-2. Cumulative patient accruals into CBCP protocols since 2002.

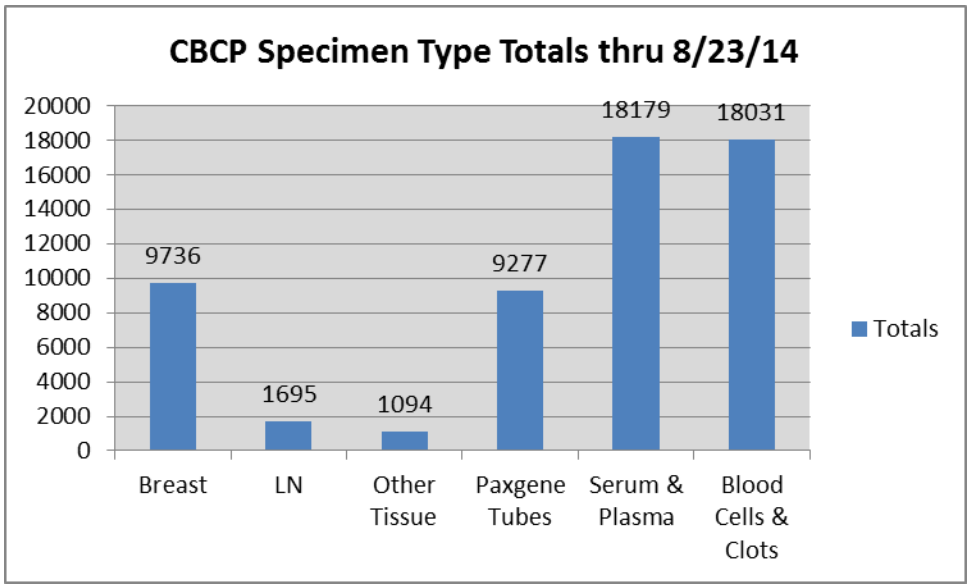


Figure BB-3. The numbers and types of biospecimens collected by the CBCP

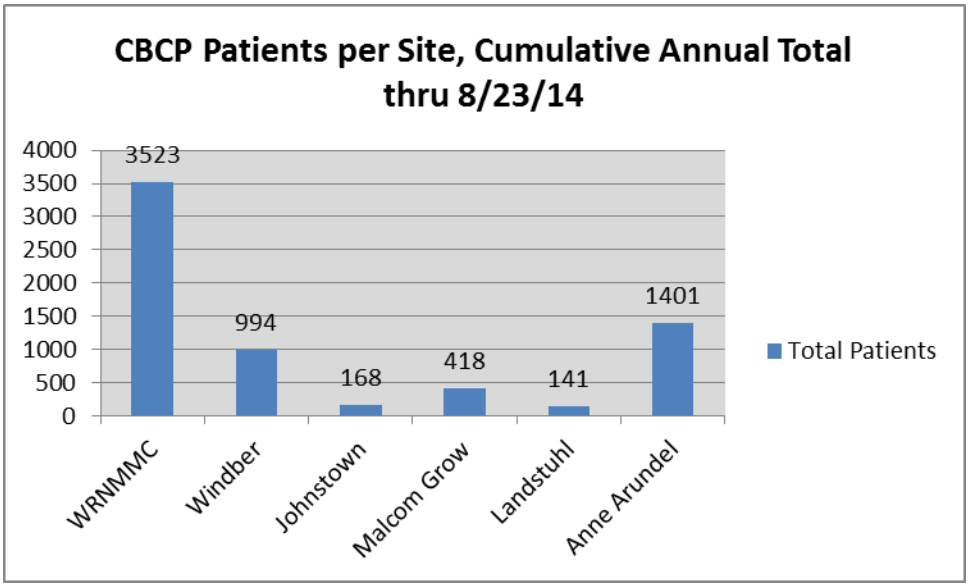


Figure BB-4. Numbers of patient recruited to CBCP protocols at various partner sites.

These specimens represent a broad spectrum of tissues, blood and blood products (**Figure BB-3**) that are not only a unique and valuable resource for the BC-TRC but are also the substrates for our translational research program. Along with the biospecimens that have been collected from CBCP participants, each consented patient also provides nearly 800 field of demographic, medical, life and family history data as well as complete pathology data on donated tissues. The collection of tissues that the BC-TRC inherits from the CBCP is even more valuable because of this rich annotation. Patients have been recruited from a number of partnering clinical intake sites over the history of the CBCP (**Figure BB-4**). At the start of the BC-TRC the active partners are WRAMC, the Joyce Murtha Breast Care Center in Windber, PA, and the Anne Arundel Medical Center in Annapolis, MD.

MEDICAL APPLICATION: One of the major challenges facing researchers and Clinicians today is understanding the mechanisms associated with the evolution of benign breast disease and/or the transition of breast disorders to breast cancer. The creation of a good and comprehensive tissue/blood bank is essential to the application of modern molecular and genetic analysis to the study of breast diseases. Amongst other goals, the BC-COE has (1) established a repository of good quality breast tissue and related specimens (lymph nodal, blood) for research on breast cancer and associated breast diseases, as well as (2) the establishment of a data warehouse with accurate and comprehensive biologically and clinically relevant information. Since the standard of care for treating breast diseases and breast cancer is based on a multidisciplinary model that integrates prevention, screening, diagnosis, treatment and management, the BC-COE project provides the necessary framework for such an integrated approach, which will positively impact the future management of breast cancer.

PLAN:

The rapid advances in basic research and data mining technologies fuels a growing demand for tissue banks. The development of new tools of molecular biology such as polymerase chain reaction (PCR), microarray and proteomic technologies, and laser capture micro dissection (LCM) has made it possible to examine gene expression in very small tumor samples and at high throughput. Tissue banks allow researchers to test their hypotheses rapidly and in a cost effective manner. By linking such molecular information to clinical data, we propose to translate knowledge from the laboratory to the clinic. These new technologies, coupled with the information that can be generated with resources available in a tissue bank, will inspire researchers in the field of breast cancer to ask questions and develop hypotheses that 10 years ago were inconceivable.

1. Subject: Subjects are consented to one or both of the Protocols; WU#01-20006 Tissue and Blood Library Establishment for Molecular, Biochemical, and Histologic Study of Breast Disease, and WU # 01-20007 Creation of Blood Library for the Analysis of Blood for Molecular Changes Associated with Breast Disease and Breast Cancer Development. Each patient is assigned a unique identifying number on entry into a study. The subject enrollment sites are the Breast Center or the Women's Imaging Center of the WRNMMC, The Joyce Murtha Breast Cancer Center [JMBCC], Windber, PA and Anne Arundel Medical Center [AAMC], Annapolis, MD. They consist of three general subject groups: 1) patients presenting with known breast cancer, 2) patients presenting with evidence of breast disease requiring clinical need for some form of tissue biopsy, and 3) patients presenting to the participating clinics for (plastic surgery) elective reductive mammoplasty. Evidence of breast disease includes potentially malignant breast lesions detected by mammography or ultrasound, palpable breast mass (es), abnormal breast discharge, abnormal physical breast morphology consistent with possible breast cancer, or axillary adenopathy without a known pre-existing condition. The majority of patients will be female, however, 1% of all breast cancers occur in males and these male patients with breast cancer, as well as males undergoing breast tissue biopsy, or reduction for gynecomastia are eligible as well. Patients are recruited at their next scheduled patient visit.

2. Inclusion and Exclusion Criteria: For consideration of inclusion in this study, all patients must meet the following criteria: 1) adult over the age of 18 years, 2) mentally competent and willing to provide informed consent, 3) military beneficiaries presenting at the Women's Imaging Center of the WRNMMC Breast Center with evidence of possible breast disease, or presenting to the clinic for (plastic surgery) elective reductive mammoplasty, 4) other patients presenting to participating sites such as the The Joyce Murtha Breast Cancer Center, Windber, PA and Anne Arundel Medical Center, Annapolis, MD with evidence of possible breast disease, or for (plastic surgery) elective reductive mammoplasty. Exclusion Criteria: Prospective participants with known history of HIV, HBV, HCV, prion-mediated disease, drug resistant tuberculosis or other infectious disease presenting a significant risk to personnel handling tissue or blood-derived products shall be excluded from participation. Prospective participants with pre-existing coagulopathies or all other conditions, for which invasive biopsy or surgery is medically contraindicated, shall be excluded.

3. Data collection: Currently a Core Questionnaire is administered to each BC-COE patient by a nurse case manager and takes from 30 minutes to one hour to complete. Data acquired includes: medical history, family history, diet, exercise, use of alcohol and tobacco, number of pregnancies, number of live births, medications, etc. The data is reviewed by data managers to insure completeness and then entered into the Clinical Laboratory Workflow System through double-data entry to ensure correctness and uniformity. For patients undergoing a biopsy, a Pathology Checklist is completed recording detailed diagnosis of breast cancer or benign lesions. The subject will be identified in the research arenas by a"BC-COE #", which will be a unique, individual patient- specific number. This BC-COE number will be linked to all patient items via barcode of samples and questionnaires. The only connection between the "BC- COE #" and the patient identifiers will be kept in a double-locked, secure file in the Office of the Director and Principal Investigator (PI) of the BC-COE at the WRNMMC location, and/or via a password-protected, secure database to which only the PI or his designee have access. There will be no way for researchers or other persons anywhere along the chain of tissue, serum, or data collection or analysis, to identify the actual identity of the patient via the barcodes or BC-COE number. Other researchers within the BC-COE or associated with the BC-COE who have permission to use the blood or tissue samples may also have access to the clinical information (indeed, this linkage is what makes the tissue bank so powerful as a research tool); however, this linkage will not involve the patient's name or any other patient identifiers, so no researchers will ever know the identity of the specimen's origin.

4. Biospecimen collection: For patient consenting to the Blood and Tissue protocol, surgical surplus tissues are collected and preserved in FFPE or OCT blocks for research use. Blood samples are also drawn for enrolled patients and processed as specified in the protocol. See Methodology.

5. Specimen repository storage – All specimens are held in the BC-COE freezers for an indefinite period of time. Exceptions to this will occur when specimens are exhausted (consumed) due to their use in the approved research within BC-COE labs and

elsewhere, or when previously consented patients withdraw their consent, at which time that patient's specimens will be withdrawn from the repository and destroyed. The BC-COE PI is responsible for maintaining the integrity of the Biorepository (Tissue Bank and Serum Bank) and all databases. In the event that the named PI leaves WRNMMC, another WRNMMC investigator will be named (through in-place processes as stated in the BC-COE charter), and that new WRNMMC BC-COE PI will take full responsibility for the banked samples and data. If under any circumstance a new WRNMMC BC-COE PI is not named, then the WRNMMC Human Use Committee (HUC) will be notified, and the HUC will determine the disposition of this tissue and blood library.

6. Sample Size Estimate: No sample size estimate is required because subject enrollment and tissue collection process is open-ended. However, we do have an expected (estimated) number of patients per year that will be enrolled at WRNMMC in the BC-COE protocols. We estimate that the number of patients newly diagnosed with breast cancer and treated at Walter Reed will average about 200 per annum. The number of patients undergoing some or any form of a breast biopsy for any reason will average about 1100 per annum.

METHODOLOGY:

1. Sample collection: The approach to collection of the samples to be archived is as follows, based on the grouping of the patients presenting.

a. Patients already diagnosed with breast cancer. This group consists of patients who already have undergone a breast biopsy of any type, at WRNMMC or the other associated institutions (after confirmation of the pathology diagnosis at that institution), which has confirmed a cancer diagnosis that requires further surgical therapy as per the consensus recommendation of the multidisciplinary breast conference. Up to 20cc of blood will be obtained from a peripheral venous access line that has been placed for the administration of anesthetic or fluids. Once the breast tissue is surgically removed, as clinically indicated, the specimen(s) will be taken to the pathology laboratory where a licensed pathologist will ensure that the tissue is adequate for routine pathology analyses (diagnosis, margin status assessment, and other indicated purposes). If appropriate, a fine needle aspiration (FNA) utilizing a 22 gauge needle / syringe setup will be performed on the breast and/or lymph node specimen(s), and the cytologic contents placed in standard solution, centrifuged, and flash frozen prior to placing them in the freezer. Then and only then, if any actual excess tissue (cancerous or benign) remains, samples of that tissue will be harvested for archiving in the tissue bank. This archival tissue will be divided and placed into vials, labeled with a code after all patient identifiers are removed, and flash-frozen in liquid nitrogen. It will then be placed into the BC-COE Tissue Bank freezer at temperatures down to –180C. Blood samples will be centrifuged prior to separation and flash freezing of the serum and non- serum components, and placed into the –180C freezer.

b. All consenting adult patients presenting to the Breast Center or the Women's Imaging Center at WRNMMC and the other associated sites with evidence of breast disease for which a breast tissue biopsy (to include ductal lavage, open breast biopsy, minimally invasive, tru-cut biopsy, image-directed biopsy) is clinically indicated. After consent is obtained, patients will

fill out the standard questionnaire and then be taken for the procedure or surgery. Up to 20cc of blood will be obtained from a peripheral venous access line that has been placed for the administration of anesthetic or fluids. If no IV access is clinically indicated, consenting patients will have the blood drawn using standard sterile techniques from a peripheral vein. Once the breast tissue is surgically removed, as clinically indicated, the specimen(s) will be taken to the pathology laboratory where a licensed pathologist will ensure that the tissue is adequate for routine pathology analyses (diagnostic, margin status assessment, and other indicated purposes). If deemed appropriate, an FNA utilizing a 22 gauge needle / syringe setup will be performed on the specimen, and the cytologic contents placed in standard solution, centrifuged, and flash frozen prior to placing them in the freezer. To clarify, this will be on a specimen already removed from the patient, now in the hands of the pathologist, who will take a 22 gauge or equivalent needle on a syringe and make several passes into the biopsy specimen in order to retrieve and store individual cells (cytology). This in no way will negatively affect the actual standard pathologic analysis. Then, if any actual excess tissue (cancerous or benign) remains, samples of that tissue will be harvested for tissue archiving in the tissue bank. This archival tissue will be divided and placed into vials, labeled with a code after all patient identifiers are removed, and flash-frozen in liquid nitrogen; it will then be placed into the BC-COE Tissue Bank freezer which has temperatures down to –180^{o}C. Blood samples will be centrifuged and separated into plasma, serum, clots and cells. These products will be flash frozen and stored at –180^{o}C.

c. Consenting adult patients presenting to the WRNMMC plastic surgery clinic for elective reductive mammoplasty. This group will consist of patients who seek elective reductive mammoplasty. These are patients who still desire elective reductive mammoplasty, after all routine clinical measures of mammography (if indicated) and clinical breast examination are found to have no contra- indication to said procedure, and have been appropriately counseled by a licensed plastic or general surgeon. After consent is obtained, patients will fill out the standard questionnaire and then be taken to surgery. Up to 20cc of blood will be obtained from a peripheral venous access line that has been placed for the administration of anesthetic or fluids. Once the breast tissue is surgically removed, as clinically indicated, the specimen(s) will be taken to the pathology laboratory where a licensed pathologist will ensure that the tissue is adequate for routine pathology analyses (diagnosis, margin status assessment, and other indicated purposes). If any actual excess tissue (cancerous or benign) remains, samples of that tissue will be harvested for tissue archiving in the tissue bank. This archival tissue will be divided and placed into vials, labeled with a code after all patient identifiers are removed, and flash-frozen in liquid nitrogen. It will then be placed into the BC-COE Tissue Bank freezer at 180C temperatures. The blood samples will be processed, and its by-products flash frozen and eventually sent to the offsite repository for storage in –180C freezer.

2. Sample handling

a. All specimens described in sections a, b and c will remain in the freezer at the WRNMMC site for at least a period of two weeks, or longer if needed to fulfill the requirement set in section (b) below. During this time, no analyses will be performed on the specimen – this period of time will be known as the "Fail-Safe" time period. The Fail-Safe time period is intended to allow the diagnostic pathologists the opportunity to withdraw banked tissue for any additional diagnostic testing they determine is necessary

for the care of the patient.

b. After the Pathologist determines with final certainty, by the publishing of the official final pathology report with no outstanding addenda, that there is no diagnostic pathology requirement for the frozen specimen(s), then the archived specimen(s) on that patient (identified only by code with the logbook as noted above) will be released to the BC-COE for research analyses. This will include transfer of the tissues to WRI for specialized studies (molecular, genomic, proteomic and histological analyses).

c. Processed blood (serum, plasma, blood clots and blood cells) will be labeled appropriately with no identifiers and linked only to the patient via the codebook. These specimens will be transferred to the BC-COE offsite research facility (WRI) for experimental analyses and storage. The cytologic aspirate when applicable will likewise be transferred to the BC-COE offsite research facility for molecular, proteomic/genomic and/or histopathologic analyses. These will be labeled appropriately, linked only to the patient via the codebook. All patient identifiers will be removed and all specimens sent to the BC-COE repository will be identified by codes.

3. Primary uses of the tissue and serum specimens.
a. Tissue Banking – this includes sample definition and receiving, flash freezing/labeling(putting identifier codes on each tissue sample for subsequent tracking)/storage, embedding in Optimal Cutting Temperature (OCT) [placing the tissues in a special preservative that protects the RNA/DNA during prolonged freezing], and inventory/tracking. The inventory and tracking of all samples is done electronically with barcodes and sample tracking software, The Laboratory Workflow System (LWS) from Cimarron Software. This tracks all genomics and proteomics experiments involving microarray, genotyping, DNA sequencing and 2-proteomic analysis.

b. BioImaging/Microscopy – after sample definition, receiving, and fixation and/or embedding, appropriate pathological slides are further subjected to Fluorescence in-situ hybridization analysis (FISH), Hematoxylin and Eosin analysis (H&E– a standard dye used in pathology to stain certain cell components and structures certain colors, to facilitate visual microscopic identification), and IHC (Immunohistochemistry) analysis. Laser capture micro dissection (LCM) is performed by trained technicians (histotechnologits) who work closely with the BC-COE pathologists. Nucleic acids are isolated from laser captured material and may be utilized for cDNA synthesis, microarray analysis, and In-situ reverse transcription polymerase chain reaction (RT-PCR). Image acquisition is performed on digital microscopes and images are archived in the BC-COE server(s) and/or data warehouse. The current data ware house has been developed in collaboration with InforSense Ltd and it currently houses the clinical data with an On-Line Analytical Processing tool (OLAP).

4. Administrations: As a multi-center protocol, for each participating site a Principal Investigator (PI) is responsible for overseeing the protocol, conforming the protocol and consent form to their own institution's IRB requirements, and for achieving their own institution's IRB approval prior to proceeding.

III. Focused Research:

There are two themes for BC-COE research. Theme 1 focuses on breast cancer mechanistic studies of clinically important questions, and Theme 2 focuses on therapy-relevant molecular studies of breast cancers.

For Theme 1 studies, one important topic is integrative profiling of breast cancers. The current 4 major breast cancer subtypes—termed "intrinsic subtypes"—were based on gene expression profiling. IHC-based subtyping using ER, PR, Ki67 and HER2 are available and are of clinical significance, although such subtyping is sometimes referred to as surrogate for intrinsic subtyping. Information on a broader panel of proteins and their post-translational modifications as well as their subcellular location information is needed for a more comprehensive understanding of breast cancer stratification which is important for cancer treatment. Thus such studies are important not only for Theme 1 but also for Theme 2, for example, the identification of protein markers for endocrine resistance.

For Theme 1 studies, the BC-COE provides a good research environment on young breast cancer patients and African American patients. Young age at breast cancer diagnosis and being African American are considered risk factors for poor outcomes of breast cancer patients. BC-COE has enrolled a high percentage of AA patients, and there is also a good size of young breast cancer patients enrolled due to the demographics of the active-duty military population. Using these resources BC-COE scientists have conducted molecular studies, and have proposed additional molecular, epidemiologic, and comparative survival analysis using both BC-COE data and the data in the public domain.

The topic of tumor heterogeneity is not only important to the understanding of breast cancer development (Theme 1), but also of therapeutic significance (Theme 2). Tumor heterogeneity refers to the cellular heterogeneity of tumor development environment, where there are cancer cells, stromal cells, lymphocytes, etc., and the MCC has chosen "Inflammation, Infection, Immunity, and Stroma (I3S) as one of the focuses for research. Tumor heterogeneity also refers to the fact that one physical tumor could contain multiple lineages of tumors that are not necessarily of the same molecular subtype. When only one subtype was diagnosed and treated, the other subtypes could be left untreated which could lead to detrimental outcome of the patient. Additional topics are proposed to be studied on mechanistic understanding of breast cancer development. These include genetic dispositions, exposure to environmental risks, access to healthcare and treatment disparities, and impact of certain life style factors as well as comorbidities.

For Theme 2 studies, profiling of human biospecimens alone is important but insufficient; biospecimens are no longer alive after excision from the human body, and in order to study the impact of drugs or the response to drugs of a mutated gene, a live model system is needed. BC-COE scientists has developed tissue culture systems for both 2D and 3D model systems of breast cancer cell lines, with a focus on the triple-negative subtype that are currently difficult to treat. Findings from such studies are validated or sometimes guided by bioinformatics analysis of the data on human tissues.

The ultimate goal of all BC-COE research projects is to generate new knowledge that will benefit breast cancer patient treatment. The large volume of molecular data from BC-COE patients, integrated with the clinicopathologic data including the highly valuable treatment and outcome information, provides a gold data mining opportunity for BC-COE scientists to generate new hypotheses for study and validate new experimental findings. This opportunity is even more enriched by the availability of large-scale high-quality datasets such as those from TCGA across multiple cancer types. Such raw data, combined with public annotation databases on genes, proteins, pathways, and human diseases, will enable derivation of new knowledge for breast cancer patient treatment.

IV. Biomedical Informatics:

As one of the five pillars of the CBCP, Biomedical Informatics (BMIX) has developed a comprehensive informatics system supporting the activities in all of the other 4 pillars. Biomedical Informatics also provides support to other research projects and leads its own research, by working with scientists both within and outside of the WRI. The team members and the system have made significant contributions to The Cancer Genome Atlas—Breast Cancer (TCGA-BC) project[1], and the Director has won several MCC Collaborative grants and the MCC-NCI Activation Funds grants, serving as a Co-PI or Co-Investigator. The team has also been tasked to lead the development of the MCC Informatics Infrastructure.

In the recent years, the BC-COE has been conducting or participating in several large-scale molecular studies, including the TCGA-BC, Massive Parallel Molecular Processing in collaboration with the Pacific North Western National Lab, a Komen Promise Grant for therapy relevant molecular stratification of breast cancers in collaboration with Thomas Jefferson, etc. New initiatives are in development. The BC-COE is now also addressing the collection of treatment and outcome data for invasive cancer patients enrolled in the study. These projects, combined with the research conducted by scientists at the WRI, has generated a large amount of molecular data as well as new types of clinical data. It is thus critical to expand our current informatics infrastructure to manage all these data, and more importantly, it is critical we expand our bioinformatics research capability to conduct integrative analysis to analyze these data, mine for new hypothesis for validation both computationally and experimentally, so as to make the best use of the data towards making important findings in understanding cancer development mechanisms, identifying cancer treatment drug targets, and develop physician decision support system to aid in cancer treatment.

Biomedical Informatics is now broadly defined as a multi-disciplinary subject for the management and utilization of biomedical information encompassing clinical informatics, public health informatics, and bioinformatics [2]. This definition is increasingly important as new concepts and technologies enter into medical practice and related basic research, and require new types of information management and data analysis that relies on sophisticated statistical and computational technologies. Figure DD.0 shows the major components in this definition of BMIX [3].

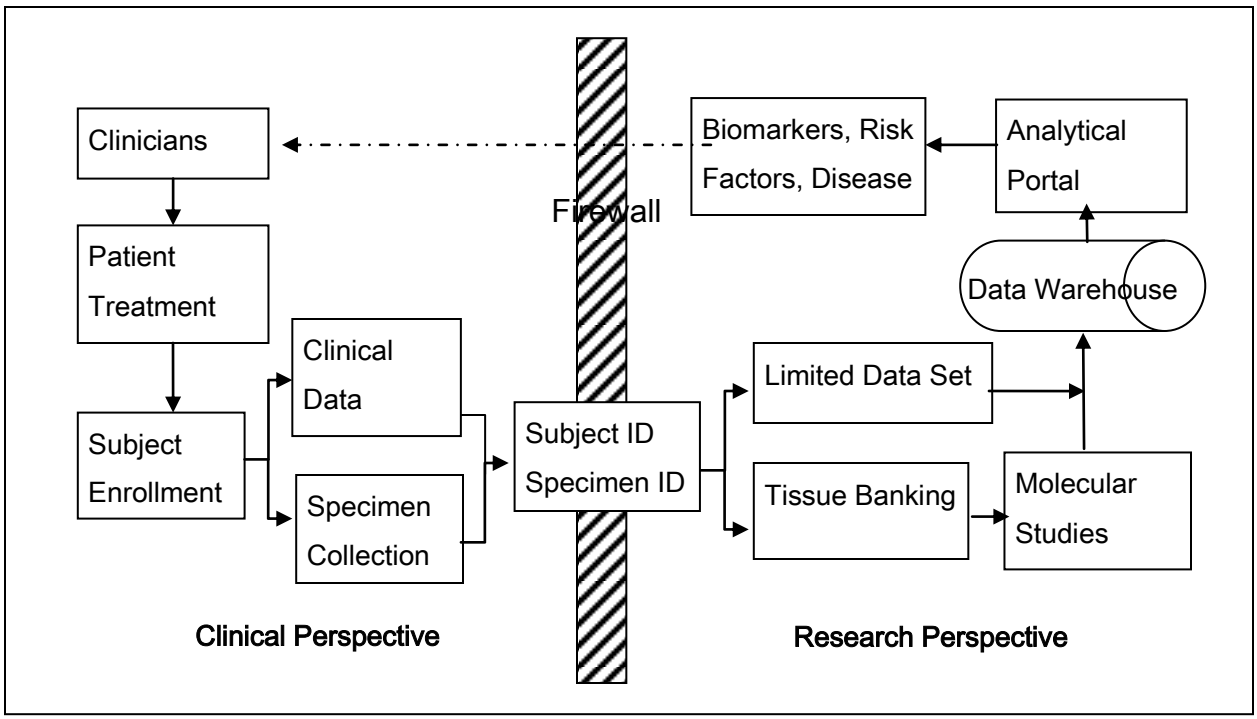


Figure DD.0. Major components of biomedical informatics. Clinically, patients receive treatment, subjects are enrolled in the study, and clinical data as well as specimens are collected. To protect the privacy of human subjects, de-identified subject IDs and specimen IDs are created and properly mapped before being transferred to the research side with the corresponding clinical data and the specimens. On the research side, clinical data are properly stored, tissues properly banked and genomic and proteomic studies conducted. All data are then warehoused, analyzed, and mined for biomarkers, risk factors, and disease models. Newly obtained knowledge is fed back to the clinic to aid in clinical decision-making.

From the data flow point of view, these BMIX components include 1) supporting data collection and generation across clinical, genomic, and proteomic platforms, 2) data tracking, 3) data centralization, 4) data analysis and mining, and 5) knowledge generation and presentation to research and clinical applications. We have been working towards developing a complete BMIX infrastructure for the BC-COE. The system we are developing was designed to be flexible to enable expansion to support translational research in other disease areas. In the following we will present the background, the current status, and the plan for each of these 5 components of BMIX.

V. Translational Clinical Care:

The objectives of the Clinical Care Pillar are to:

a) Decrease the negative psychological impact on the patient of having an evaluation or treatment intervention for breast disease by utilizing objective measurement instruments to longitudinally assess the patient's psychological response to evaluation and intervention, and base modifications of these procedures on those results.

b) Create and maintain an environment (medical, physical, psychological) conductive to the multiple needs of the patient undergoing breast disease evaluation / treatment.

c) Recruit patients into the various BCTR protocols to obtain the clinical data and biospecimens needed to meet the BCTR's translational research goals.

This pillar of the BCTR is the foundation upon which all the success of and project rests. Without patients enrolled in our biospecimen repository protocols, there would be no translational research center of excellence. These patients come from the clinical care environment. Since its inception in 2000, the CBCP (now the BCTR) has had as a priority, the development and staffing of the core clinical centers at Walter Reed National Military Medical Center, the Joyce Murtha Breast Care Center in Windber, PA and at our newest site, the Pat and Lesly Sajack Breast Center at Anne Arundel Medical Center in Annapolis, Maryland. Under the direction of Lorraine Tafra, MD more than 500 newly diagnosed cases of breast cancer are seen at AAMC each year.

At each center the staff is dually trained as clinical/research providers, to seamlessly integrate the need for a strong research focus in the clinical center with the requirement to provide state-of-the-art clinical care to the patients.

The reputation of the BCTR is that of an exceptional translational research project with very possibly the world's most pristine collection of breast tissue. This has resulted in a number of well-respected medical centers expressing interest in joining us as research partners.

The care of our patients is provided by Physicians, Advance Practice Nurses (Nurse Practitioners) and certified Nurse Navigators with all personnel having as their prime job description, the research aspects of the BCTR.

Walter Reed National Military Medical Center in Bethesda, MD has a state of the art comprehensive breast care center with women's imaging co-located with the breast care center. The Breast Center has a procedure room and recovery room enabling surgery within the center. The Breast Imaging Center has a designated Aurora Breast MRI machine. The Breast Care and Translational Research Center of Excellence received a 3 year full accreditation by the NAPBC (National Accreditation Program for Breast Centers) on September 11, 2012, which makes BCTRCOE accredited through September 2015. We anticipate seeing between 7,500 patients per year and diagnose approximately 250 new breast cancers per year.

There is a multidisciplinary conference held every Thursday for newly diagnosed breast cancer patients and their significant other(s) are assigned an examination room where they meet the various providers that comprise the interdisciplinary breast care team. Providers include a breast surgeon, a medical oncologist, a radiation oncologist, a psychologist, nurse navigators/case managers, a physical therapist, and a plastic surgeon. Each specialty has the opportunity to meet privately and evaluate each patient. The benefit of this approach is that instead of having individual appointments spread over several days or weeks, the patient need only schedule one day to see all the various providers.

This allows us to educate, facilitate and coordinate a comprehensive breast treatment plan for the

patient that maximizes treatment options and streamlines patient care in a patient-focused environment. It also allows us to discuss the various research protocols with patients and, if they agree, obtain informed written consent and complete, with the assistance of a research nurse, an extensive clinical questionnaire that captures clinical data.
After morning activities, the breast care team assembles and conducts an interdisciplinary conference to discuss each patient's case, resulting in a comprehensive treatment plan built on a team consensus. The results of the conference are then reviewed with the patient/significant other and time is provided to clarify and ask questions.

Psycho-Social Oncology Services:
The WRNMMCB has 2 designated psycho-oncology staff members (1 psychologist, 1 social worker) to assess all newly diagnosed breast cancer patients. The yearly rates average about 110 new patients. These staff members also evaluate and counsel other cancer patients throughout the hospital system.

Services provided for the breast center:

- Psycho-social assessment including NCCN distress scale for all new breast cancer patients
- Individual, couples, family counseling related to diagnosis and other stressors. Telephone support also available for those for whom transportation is an issue
- referral to community resources as needed
- multi-discipline collaboration and coordination of care

Groups offered:

- Breast Cancer support group meets 2x/month 1.5hr each-open to all stages and ages occasional guest speakers
- Breast Cancer yoga class meets 2x/month 1hr each session-all stages, ages, and abilities
- Young women's (ages 20-40) Cancer (all cancers) educational support group 8 week sessions 1.5hr ea.

The Multidisciplinary Conference

At the Walter Reed National Military Medical Center, we expect to see approximately 10,000 patients per year and will diagnose approximately 200 plus new breast cancers per year.

The Multidisciplinary Conference occurs every Thursday and the purpose of this day is to Provide an opportunity for the newly diagnosed breast cancer patient to meet all the providers that comprise the interdisciplinary breast care team. Providers include a breast surgeon, a medical oncologist, a radiation oncologist, a psychologist and /or social worker, nurse navigators/case managers, a physical therapist, and a plastic surgeon. Each specialty has individual private appointments to assess and evaluate each patient who, with significant others of their choice, is given a private room for the day. The benefit of the Multidisciplinary Conference Day is a one day visit to see all the various providers instead of having individual appointments spread over several days or weeks.

This allows us to educate, facilitate and coordinate a comprehensive breast treatment plan for the patient that maximizes treatment options and streamlines patient care in a patient-focused environment.

It also allows us to discuss the various research protocols with patients and, if they agree, obtain informed written consent and complete, with the assistance of a research nurse, an extensive questionnaire that captures several fields of clinical data.
The breast care team assembles and conducts an interdisciplinary conference to discuss each patient's case, resulting in a comprehensive treatment plan built on a team consensus. The results of the conference are then reviewed with the patient/family and time is provided to clarify and ask questions.

Military Relevance:

Breast cancer is the most common non-skin cancer in women. It is the single greatest cause of cancer deaths among women under 40, and is a significant cause of mortality for women in the United States Armed Forces. Breast cancer mortality among women <50 years accounts for >40% of years of life lost due to this disease. The economic, social and emotional costs to families are far greater when a young woman dies than when an older woman dies of breast cancer. The more aggressive nature of the disease in young patients along with the attendant costs underscores the importance of early detection of breast cancer in young women. Breast cancer is a curable disease if it is detected early; as such early detection is related to survivorship, cost of treatment and quality of life for the affected woman.

The majority (>90%) of women in active military service are < 40 years of age. The Department of Defense (DOD) with its high percentage (and increasing percentage, as all roles in the military are now open to all genders, including combat roles) of young women and its commitment to health care is particularly concerned about breast cancer. When discovered at a later stage, treatment of breast cancer is expensive, aggressive and results in considerable disruption to the woman's ability to contribute to the military and society. Cost and disruption to life are considerably less when the carcinoma is discovered at an earlier stage and therefore treatable with less invasive methods and curable in up to 90% of cases for Stage I disease. Furthermore, the DOD has a high percentage of African-American (~30%) and Hispanic (~10%) women. Death rates from breast cancer tend to be particularly high in these ethnic groups owing in part to later stage of detection and to the more aggressive nature of breast cancer in these groups.

The active duty military force is approximately 20% female. Most of these service members are in the age range (30-40 years) where routine screening for breast cancer consists only of clinical breast examination. Both mammography and clinical breast examination have a very poor accuracy in the young active duty force in determining which breast abnormalities require treatment, and which are benign and can be left alone. The immense scale and impact of this problem for the military can be assessed by the fact that there were over 2,000 cases of breast cancer diagnosed in active duty service members over the last ten years (source: ACTURS DoD Tumor Registry data).

Furthermore, there were over 8,000 unnecessary breast biopsies done on active duty women during this time because it takes 4 breast biopsies of normal non-cancerous lesions to find each individual breast cancer. Hence, women often need to take lengthy amounts of time off from duty in order to undergo multiple tests leading up to the biopsy as well as time off from duty because of the biopsy itself. This translates into approximately 10,000 weeks, or 30 person-years, of time lost in the evaluation of normal, benign breast lesions in active duty service members. This would be unacceptable for any other healthcare issue, and should be so for this one. Unfortunately, at the present time there is no completely accurate screening tool currently available to diagnose breast cancer in the early, curable stages for women under the age of 40, who make up the vast majority of women in military uniform.

As indicated, approximately 20% of the active duty military force is female, most under the age of 50. Breast cancer strikes one in eight women in her lifetime, and there is a documented change in breast cancer incidence in recent years, such that breast cancer is being detected and diagnosed more often in younger women (under age 50), and the same is true in our military members. In the same way that diagnostic and therapeutic efforts through the military and US Army are carried out in infectious disease care and research, eg. Malaria, Typhoid, etc., so too must the military continue to address the effects of the scourge of breast cancer and breast diseases on the 20% of total active duty force who are women.

Moreover, CBCP, now the BC-COE, developed and to this day maintains the only specialty breast cancer evaluation and treatment center in the US Army, which is at the CBCP Comprehensive Breast Center at Walter Reed National Military Medical Center.

Additionally, ours is the only Army facility that financially supports direct genetic testing of active duty (all Services) women who are identified in our Center as being in a high risk category of carrying a BRCA genetic mutation, which when present can signify an up to 90% increased risk of breast cancer development, and for which we then deploy individualized cancer preventive therapies.

The BC-COE (CBCP) Breast Center is the Army-recognized and Military-recognized specialty referral center for tri-service active duty personnel from around the globe with medical disorders related to all breast diseases and breast cancer. CBCP Breast Center routinely cares for women on active duty Army from places such as the Middle East, Southwest Asia, OEF, Korea, Europe, and the Far East. CBCP at WRNMMC annually cares for over 7,000 patients.

Public Purpose:

The foundation of the BC-COE, the CBCP, has been in existence for over fourteen years. Its uniqueness and excellence is well known and has been attested to by numerous world-class cancer research experts, and from the large number of public and invited presentations we have given over the last 14 years. BC-COE researchers also have an extensive peer-reviewed publication and scientific communication record, now numbering in the hundreds of scientifically-validated contributions. This proposal is an application that continues and refines/focuses this established, unique effort.

The BC-COE has the world's largest biorepository of highly-characterized and pristinely-collected specimens from breast patients made up of human breast tissues, lymph nodes, sentinel nodes, sera, bone marrow aspirates, cancers, benign tumors, and pre-malignant disease, which amounts in-total presently to 58,012 specimens as of 23 August 2014. This unique DoD resource, stored, maintained, tracked, and kept under strict QA in the CBCP-contracted repository at the Windber Research Institute since 2001, is used by both internal genomic and proteomic researchers, as well as for targeted collaborations with extramural collaborators from academia, governmental organizations, and corporate entities.

This biorepository is also unique in that its specimens are tightly coupled to highly-accurate clinical, demographic, and pathologic data collected from its originating patients through robust IRB-approved and fully HIPAA (Health Insurance Portability and Accountability Act)-compliant protocols that exceed all existing regulatory requirements for patient consent, privacy, and oversight.

The BC-COE has one of the few fully integrated genomic and proteomic molecular biology research programs in the nation devoted exclusively to research in breast diseases. We have an established track record of publication and scientific communication in this field.

The BC-COE has deployed a unique biomedical informatics data warehouse system that integrates clinical, pathologic, and molecular data on breast research subjects, allowing for a novel in-silico biology discovery platform.

The BC-COE is a true translational research-clinical care environment, where there actually exists an organizationally-driven and structured collaborative effort between basic scientists, clinical scientists, clinicians, nurses, patients, and multiple other personnel.

The BC-COE has successfully expanded to other clinical sites and has established other research collaborations with world-renowned lab researchers.

III. Key Research Accomplishments

Breast Cancer Translational Research Center of Excellence (BC-COE) Statement of Work

Task 1: Identify and counsel 100 patients annually at high risk for development of breast cancer, and employ risk reduction strategies.

- See page 10

Task 2: Accrue over 500 patients annually to the "core" BC-COE protocols through consenting patients in the main BC-COE clinical sites.

- See page 14

Task 3: Acquire through consented protocol acquisitions, over 5,000 specimens annually (neoplastic and non-neoplastic breast tissues and tumors, lymph nodes, metastatic deposits, blood and its components, bone marrow) on patients with all types of breast diseases and cancer.

- See page 13

Task 4: Bank these biospecimens in the BC-COE Biorepository as the substrate for all molecular analyses carried out in BC-COE labs, as outlined in the BC-COE Core Protocols. Utilize this repository as the basis for intramural and extramural collaborations for secondary usage research.

- See page 13.

Task 5: Perform focused research as outlined below on the biospecimens and clinical data collected under the BC-COE Core protocols including global expression analysis of the DNA, RNA, and Protein features and including targeted research into genomic analysis of Stages I, II, and III breast cancer, DCIS, LCIS, and pre-malignant neoplasia.

- Present findings in peer-reviewed national meetings and publications, see page 39-41.

Task 6: Perform whole genome DNA sequencing on DNA from 40 or more cases of breast cancer over the life of the project.

- There was no new case analyzed.

Task 7: Develop and support a robust laboratory information management system to ensure proper tracking of data acquisition and a clinically relevant and laboratory research-linked prospective, longitudinal computerized data warehouse to support translational research and ultimately support physician decision making. Ongoing

- We have started designing and developing a new tracking system to replace the current CLWS system that is in place. A detailed design document has been created by using existing knowledge of the current CLWS system and by gathering requirements from stakeholders. We started creating a web-based application based on our detailed design. This system uses Oracle as the back end; the interface was developed in Java and hosted on an Apache Tomcat web server. Using this new technology, we finished designing many of application screens. We have made a strategic decision to utilize FreezerWorks functionality for tissue processing and handling as part of the new LIMS system.

Task 8: Develop an analytical system for integrative data analysis and mining, and develop a breast knowledgebase to support clinical and research activities in BC-COE. Ongoing

- The process for updating the data warehouse information was improved in several ways. One improvement was redesigning the way that historical information is archived, eliminating the necessity of making manual updates to the data workflows with every new data load. A second effort was aimed at automating the data warehouse loading process as much as possible, streamlining the process and eliminating a potential source of data errors. In addition, a Tissue Experiment Inventory was designed and developed so that experimental results could be archived in a way that was consistent and searchable. This allows future research to benefit from prior experimental results and minimizes the necessity of rerunning experiments on a finite tissue supply. Existing legacy experimental results information was cataloged, organized, and entered into a database, and an application was

developed to allow future experimental results to be entered directly into the database and allow queries against the database.

Task 9: Conduct quantitative analysis of therapy relevant proteins by immunohistochemistry within subclasses of breast cancer to provide better patient selection into clinical trials for targeted and combination therapies.

- A proof of principle of the analysis for 27 markers have been performed for over 200 cases, connecting the information on ER, PR, and HER2 to other markers for increased understanding of molecular connectivity in breast cancer tumors. Analysis of one marker CD163 has been performed with has been shown to be associated with patient survival and breast cancer subtypes.

Task 10: Study molecular differences between breast tumors from African American and Caucasian women as the identification of such differences will allow for the development of more effective therapies that will improve outcomes in African American women with breast cancer.

- Complete

Task 11: Using state-or-the-art 3D cell culture techniques and modern approaches to the study of cancer cell biology, study the mechanisms of cell invasion, migration and ultimately metastasis in breast cancer cell lines. Ongoing, several abstracts and publications have been presented on this topic.

Aim 1. CSPG4-NEDD9 interaction promotes triple-negative breast cancer progression a metastasis.

NEDD9 has been demonstrated to play a role in adhesion and migration of various cell types including lymphocytes and malignant cells. NEDD9 contains numbers of tyrosine residues phosphorylated by tyrosine kinases such as focal adhesion kinase, suggesting its role in promoting migration and invasion of cancer cells. Indeed in breast cancer progression model, previous study provided evidence that NEDD9 is a key molecule to promote breast cancer progression by facilitating migration and invasion. It is however, the mechanisms of NEDD9 in promoting progression and metastasis are not clear at present.

Given that enhanced migration and growth are hallmarks of malignant phenotypes, characterizing the mechanisms of NEDD9-mediated migration and growth provide information not only for understanding tumor biology, but also developing targeting therapies. In this study, we provide results demonstrating glycan structures (chondroitin sulfate glycosaminoglycan, CS) are altered by NEDD9 expression and regulate colony-formation and mammosphere formation of breast cancer cells. The fact that CS was not involved in promoting NEDD9-mediated migration suggests that NEDD9 would regulate malignant tumor phenotypes by multiple pathways, thereby serving as a model system for characterizing this prometastatic protein in the light of tumor biology as well as a therapeutic target in cancer therapy.

Aim 2. Development of DNA aptamers against CD44 that inhibit breast cancer invasion and metastasis.

CD44 adhesion molecules are expressed in many breast cancer cells and have been demonstrated to play a key role in regulating malignant phenotypes such as growth, migration, and invasion. CD44 is an integral transmembrane protein encoded by a single 20-exon gene. The diversity of the biological functions of CD44 is the result of the various splicing variants of these exons. Previous studies suggest that exon v10 of CD44 plays a key role in promoting cancer invasion and metastasis, however, the molecular mechanisms are not clear. Given the fact that exon v10 is in the ectodomain of CD44, we hypothesized that CD44 forms a molecular complex with other cell surface molecules through exon v10 in order to promote migration of breast cancer cells.

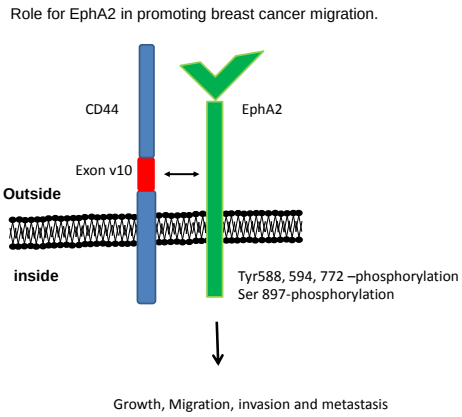
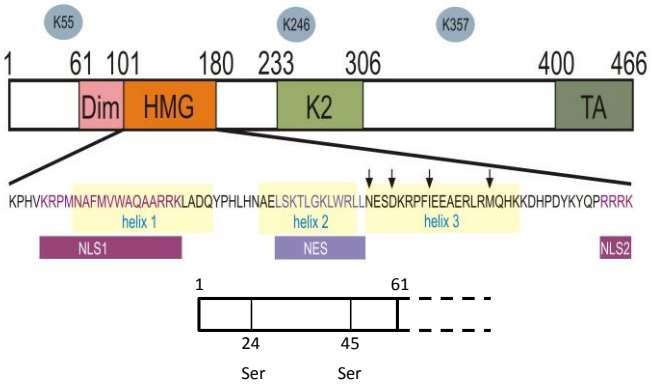


Figure 1: Working Model for CD44-EpHA2 interaction to promote TN breast cancer invasion and metastasis

We demonstrated that EphA2 was co-precipitated with CD44 and was pulled down by exon10 peptide-conjugated beads, suggesting that CD44 forms a molecular complex with EphA2 through exon v10 on breast cancer cell surface. EphA2 is a receptor tyrosine kinase for Ephrin1 and plays a key role in promoting progression and metastasis of various tumor cells including breast cancer. Our results suggest a novel model of molecular complex consisting of CD44 and EphA2 that promote of tumor progression and metastasis (Figure 1). However, there are still questions regarding the mechanisms of the formation of the molecular complex; whether the complex is pre-formed on the cell surface or the ECM-ligand induces the formation on the cancer cell surface, which is a key question for understanding the signaling pathways that promote cell migration.

Aim 3. Identification of drug-targets for triple-negative breast cancer.

We developed systematic approaches to identify drug targets by encompassing Biology, Bioinformatics, and animal models between WRI and USUHS. We are currently characterizing MIA and SOX10, which are highly expressed in triple-negative breast cancer tissues, for their biological functions in facilitating tumor growth, migration, and growth. We established a protocol for silencing SOX10 expression by using siRNA and



Potential sites for O-GlcNac modification

demonstrated that SOX10 plays a key role in promoting breast cancer migration, but not growth.

Figure 2: Domain Structure of SOX10

We are currently identifying proteins downstream of SOX10 by proteomics approaches including 2-DIGE and microsequencing target proteins. This project is also supported by USMCI-CCC grant in collaboration with Professor Mary Lou Cutler at USUHS.

We are currently evaluating two Ser residues in SOX10 at 24 and 45 for regulating breast cancer migration and growth. We constructed plasmids in which two Ser residues are mutated as fusion protein with FLAG peptide. Thus, we could express and detect the mutant SOX10 by western blotting analysis using anti-FLAG peptide. We are currently optimizing the transfection protocol using HCC38 and MDA-MB-231 as parental cell lines.

Aim4. Development of novel Ruthenium (Ru)-compounds as anti-cancer reagents.

According to the latest issue of Cancer Facts & Figures (2013), more than 230,000 women will be diagnosed with invasive breast cancer in the USA and nearly 40,000 patients will die, which ranks second as a cause of cancer death. When breast cancer cells express hormone receptors including progesterone, estrogen, or Her2/neu receptors, there are effective several treatments targeting these receptors. Triple negative (TN) breast cancer, which comprises 15% to 20% of breast cancer cells aggressively invade and metastasize to distant organs. One of the issues for TN breast cancer therapy is to develop therapy regimen for maximizing pathologic complete response rates to improve prognosis. Given the serious side effects of chemotherapeutic agents, strategies to reduce the amount of drugs administered have been considered. Thus, the immediate requirements are to develop novel synthetic compounds that inhibit tumor growth *per se* and enhance tumoricidal activity of chemotherapeutic agents.

Although ruthenium (Ru) complexes have been evaluated to inhibit growth of various cancer cells [1,2], there is limited information their effects on breast cancer cells. The goal of this proposed project is to develop Ru complexes that inhibit TN breast cancer growth and metastasis through systematic structure-activity relationship studies. We demonstrated that one Ru complex significantly inhibited growth of various tumor cells including breast cancer, osteosarcoma, melanoma, and lymphoma

Our ultimate goals are to synthesize Ru (II) complexes that effectively inhibit breast cancer cell growth. We anticipate that Ru (II) complexes would function as novel anti-cancer drugs by themselves or in the combination therapy with chemotherapeutic reagents, which would lead to a maximizing the effect of anti-cancer drugs by reducing their side-effects during therapeutic processes.

Task 12: Use our unique collection of breast cancer biospecimens to characterize microRNA (miRNA) expression in breast cancer progression and metastasis.

- This project is on hold.

Task 13: Identify protein signatures associated with the development and progression of pre-

malignant breast disease to improve our understanding of the biologic processes involved in early breast disease development and progression and to drive the development of personalized therapeutics for breast disease.

- This project is currently inactive.

Task 14: Identify genetic changes in low- and high-grade breast tumors to improve our understanding of the evolutionary process of breast cancer and to identify a protein signature that can discriminate low- from high-grade breast tumors, allowing for more accurate diagnosis and risk assessment.

- An abstract reporting the genetic results is being planned for AACR. The protein data needs to be analyzed by the incoming statistician.

Task 15: Use our unique collection of breast cancer biospecimens to characterize molecular signatures that can differentiate primary breast tumors with and without metastatic potential, as well as between primary tumors and subsequent metastases.

- We have completed two articles and another is in preparation.

Task 16: Improve our understanding of the molecular changes associated with HER2 amplification and over-expression to allow for more precise diagnosis of HER2+ patients and development of customized treatment options in patients with HER2+ breast cancer. Ongoing Objective 1 Evaluate differences in molecular profiles of patients with increased HER2 expression. Ongoing.

- Preliminary data show differences in gene expression between patients with increased HER2 expression due to HER2 gene amplification vs. chromosome 17 polysomy. Additional cases have been identified and are being evaluated for gene and protein expression differences.

- Objective 2. Determine how moderate expression of HER2 differs from no expression in patients with ER positive breast tumors. Complete

Task 17: Study the role of matrix metalloproteinases in breast cancer with the goal of developing diagnostic and prognostic marker of breast cancer based on expression of MMPs and polymorphisms in MMPs.

- Complete with a publication.

Task 18: Identify molecular alterations in the breast tumor microenvironment that contribute to tumorigenesis and which may lead to improved methods of breast cancer prevention and treatment.

- Adipose pilot study complete with publication in 2014.

Task 19: Use our unique collection of breast cancer biospecimens to study angiogenesis and lymphogenesis in different grades of DCIS and IDC.

- This project is on hold.

Task 20: Incorporate the rapidly growing public genomic and proteomic datasets related to breast cancer into our data warehouse to be able to mine the combined data sets for the generation of

new hypotheses regarding breast cancer development, progression and treatment. Ongoing, outgrowth of project with NCI/NHGRI TCGA project.

- Subaim 1. Generate a tissue-experiment inventory for TCGA-BC BC-CoE cases.
  Complete.
- Subaim 2. Integrate gene expression microarray data for both Level 1 and Level 3 data
  Complete for Level 3 and decided that Level 1 is not as important.
- Subaim 3. Develop applications to use the integrated gene expression data
  Complete at the query level and decided that more advanced applications will not be cost-efficient, and our resources are limited.
- Subaim 4. Integrate Level 3 DNA Sequencing data, and make the results available to scientists, suing similar approaches
  Complete, and same logic for applications apply.
- Subaim 5. Integrate SNP data and make the results available to scientists using similar approaches
  This type of data is not immediately needed so this subaim is on hold.

Task 21: Comparing biomarker expression in core biopsy and surgically resected tumors.

- Complete with a poster presented at SABCS 2013.

Task 22: HER2+ and HER2- luminal B subtypes of invasive breast cancers. Ongoing.

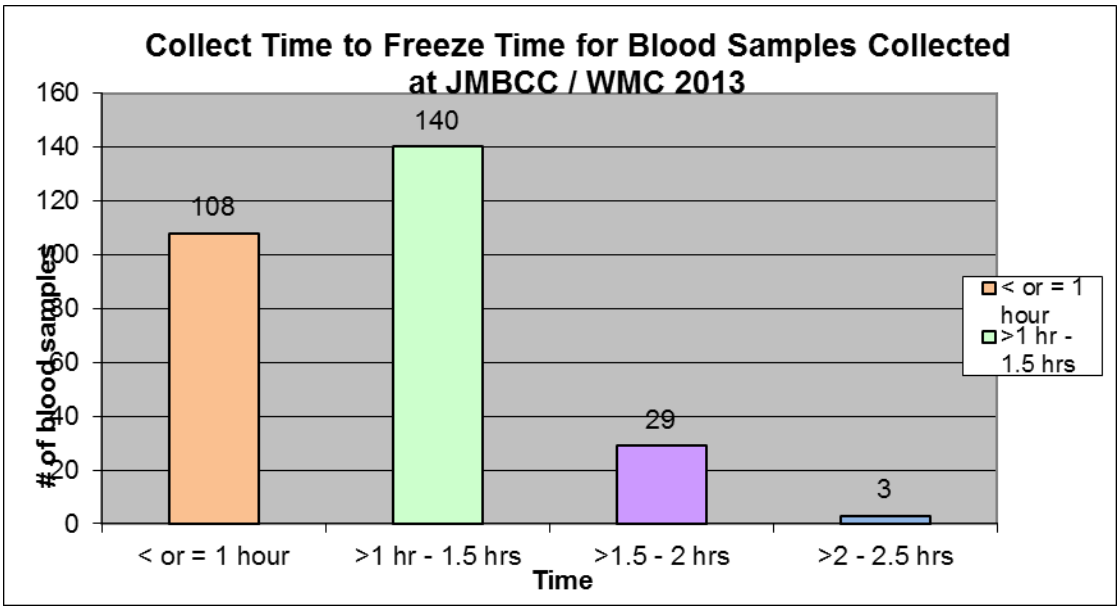
- Complete for the performance period with a poster presented at the SABCS 2013. Additional analysis will be performed as more outcome data are available towards a publication.

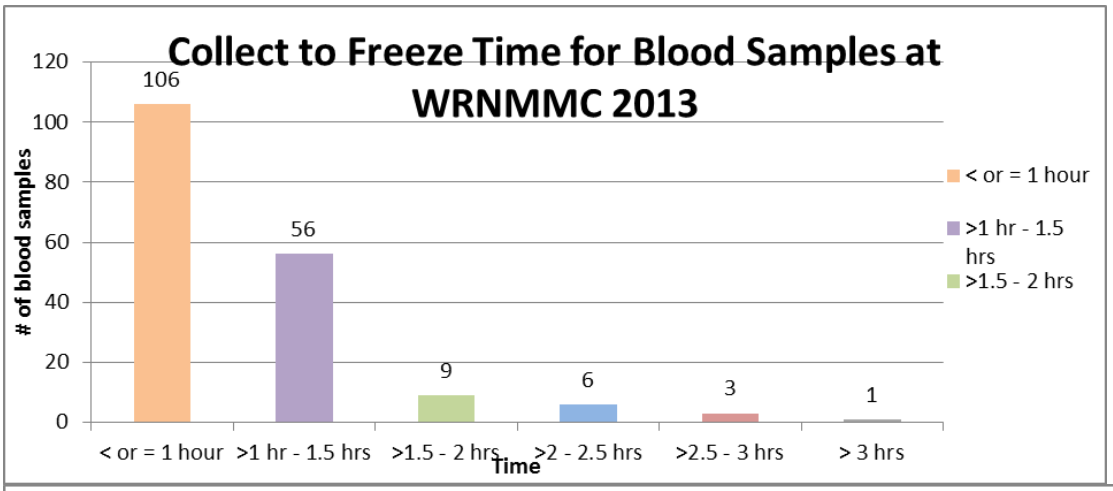
Task 23: Biospecimen research activities to evaluate the effect of a variety of pre-analytical variables on samples collected for tissue banking Ongoing

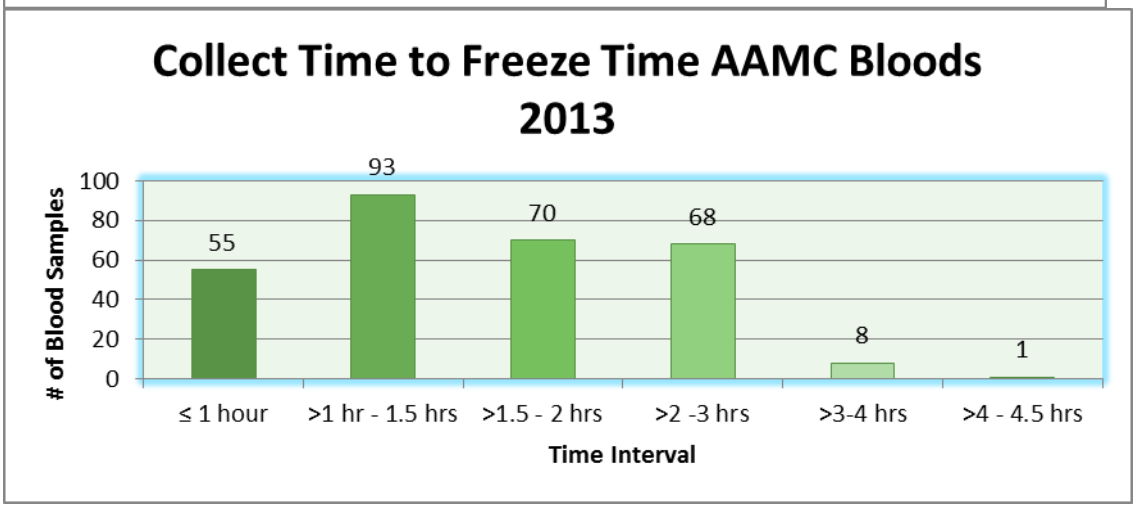
- **Documentation** Tissue integrity is essential to the results obtained from the downstream experiments for which they are utilized. The quality of the specimens will determine the ability to effectively translate these experimental results from the laboratory to the clinic. Tissue integrity is assessed from the moment it is obtained from the donor and through all the different steps which include its handling and final storage in the repository. We have put in place systems to enable us to track as much information as possible from the moment the specimen is obtained from the donor through its handling, processing and storage to enable us understand how we are working to effectively maintain currently described best practices in our repository. Some of the activities include:
  a) The time that a tissue is out of the donor is tracked together with the time the tissue is processed and stored. This includes solid tissue from surgical procedures and blood.
  b) Any deviations observed from samples from collaborating sites are documented such as short draws, shipping conditions etc.
  c) A comprehensive standardized system is in place for verification of sample receipt.
  d) All shipments require standardized forms and reports to be completed.
  e) All data entry is verified by two technicians for efficiency and accuracy.

f) Regular remote and manual temperature and liquid nitrogen monitoring of freezers are performed. This allows us to determine whether the stored samples experience any temperature changes that could affect their integrity due to freezer malfunction/failure.

- **Reporting and Analyses** Multiple Quality Assurance studies were completed for blood samples collected at Joyce Murtha Breast Care Center (JMBCC), Walter Reed National Military Medical Center (WRNMMC) and Anne Arundel Medical Center (AAMC). The purpose of these quality assurance studies is to analyze the time intervals between collection, receiving and freezing for blood samples collected in red and green top tubes during 2013. Data including donor identification number, collection dates and times, receive times and freeze times are recorded for blood samples collected from each site. This information is analyzed periodically to ensure uniformity is maintained among sites as well as monitoring that they conform to Best Practices for blood collection and processing to maintain sample integrity. These types of Quality Assurance studies help us to achieve our ultimate goal of providing high quality, well-annotated biospecimens to accelerate cancer research. Data below show summary graphs of Collection to Freeze time for blood samples obtained at the different collection sites in 2013. Ninety nine percent of blood samples collected from the Joyce Murtha Breast Care Center (JMBCC) were frozen within two hours of collection. For Walter Reed National Military Medical Center WRNMMC), 94% of blood samples were frozen within two hours of collection. Anne Arundel Medical Center (AAMC) had 74% of blood samples frozen within two hours of collection and 97% were frozen within three hours of collection. These kinds of data analysis allow us to monitor our activities and determine if corrective action is required.







- **Biospecimen Research** The tissue bank has instituted biospecimen research as part of its banking activities so that future SOPs will be evidence-based. Current best practices are based on experience that reflects the most effective approaches to the procurement and handling of biospecimen. We have designed research activities within the context of observed pre-analytical variables to generate data that will feed our SOPs and provide new evidence based biobanking standards for our repository. One of such experiments was to determine the effects of temperature and processing methods on the nucleic acid quality obtained from tissue specimens. Our observations indicate that RNA quality based on the RNA Integrity Number (RIN) is not significantly different for OCT or Flash Frozen tissue ($p = 0.48$) and the quality of tissue specimen (based on RNA RIN) left for up to 5 hours at room temperature is not adversely affected when compared to breast tissue processed and frozen immediately after excision ($p = 0.19$). However we have observed that the extra manipulation associated with Laser Microdissection significantly affects the tissue's integrity when compared to non-Laser Microdissected tissue ($p < 0.001$). Regarding the long term storage of breast tissue, our observation is that it can remain stable for up to 5 years in liquid nitrogen. These observations provide evidence for determining how best to adjust our SOPs so that we continue to provide quality tissue to feed new biomedical discoveries.

- **New tissue bank collection methods/protocols new collection methods**Our current observations over the years are that the availability of solid tumors/tissue for research continues to diminish due to size restriction of the surgical material available. Availability of good quality specimens is needed to feed biomedical research. In view of this situation, we have started to evaluate alternate sources of processing tissue for long term storage. Our first project in this area was evaluating the feasibility of adapting "touch imprints" as a way of accessing the limited surgical specimens for research purposes. The results of this study clearly showed that touch preparations on microscope slides and filter paper can produce sufficient good quality DNA even after 2 weeks of storage at room temperature. Also, the study was able to show that useful touch preparations could be made from samples that were frozen for up to 10 years. This highlighted the potential of using touch preparations to prolong the use of "rare and precious" specimens. The study led to a publication* in collaboration with our Walter Reed colleagues.

Task 24: Evaluation of molecular and epidemiological data associated with outcome disparities in African American women with breast cancer

- Objective 1: Complete.
- Objective 2: Outcome data is required from WRNMMC which has not yet been provided.
- Objective 3: Complete with negative results.

Task 25: Evaluation of molecular and epidemiological data associated with outcome disparities in Young Women with breast cancer.

- Objective 1: Demographic and path analysis was completed and presented at AACR. Treatment data not yet provided by WRNMMC.
- Objective 2: Completed. Results will be prepared in manuscript form in 2015.
- Objective 3: Completed. Results will be prepared in manuscript form in 2015.

Task 26: Identification of blood-based signatures of breast disease. Ongoing.

- Objective 1: Completed. Produced negative results.
- Objective 2: Completed. Negative results were yielded.
- Objective 3: generation of protein expression data from serum from patients with and without metastatic disease using DiscoveryMap arrays.

  This project has not begun. Currently, no agreement exists between WRNMMC and Myriad. We do hope to carry out this project however it will have to be paid for with CBCP supply funds rather than Myriad offering to run the samples for free.

Task 27: Effect of a diagnosis of invasive breast cancer on lifestyle choices.

- Objective 1. Data from lifestyle factors including fat intake, alcohol and tobacco use, exercise, BSE, HRT use and BMI will be collected from patients diagnosed with invasive breast cancer or benign breast disease who have filled out core questionnaires from baseline and follow-up visits The data collation is complete.

- Objective 2. Statistical analysis will be performed to determine whether these factors improve in the invasive group and if they improve more significantly compared to the benign group. The statistical analysis is in progress.

Task 28: Abundance and distribution of polychlorinated biphenyls (PCBs) in breast tissue.
The objective of this project is to assess the abundance and distribution of PCB congeners in human breast tissue through a comprehensive survey of mastectomy specimens from the Clinical Breast Care Project. Breast tissues have been collected from 302 quadrants from 62 patients with pathological diagnoses ranging from disease free prophylactic mastectomy samples to metastatic breast cancer. Analysis of 98 PCB congeners in these tissues has been conducted by pressurized liquid extraction followed by high resolution capillary gas chromatography. In collaboration with Paul J. Kostyniak, Toxicology Research Center, State University of New York at Buffalo.

- The manuscript is in preparation.

Task 29: Genomic heterogeneity in primary breast carcinomas and among sentinel lymph node metastases: Implications for clinical management of breast cancer patients

- The manuscript is under revision.

1. Future Plans
   Continued progress on SOW.

2. Problems/Issues:

   a. Current Problems/Issues
   See issues described in Progress Detail for specific tasks.

   b. Anticipated Problems/Issues
   None.

IV. **Reportable Outcomes**

24 August 2013 – 23 August 2014 Annual Report Numbers

Total Samples Collected
Total Blood: 2764
Total Breast: 1056
Total LN: 61
Total Other: 165

Total Patients Collected From
WRNMMC 224
Windber 63
AAMC 159

CBCP Publications 24 AUG 2013 – 23 AUG 2014

Rummel S, Penatzer CE, Shriver CD, Ellsworth RE. "PSPHL and breast cancer in African American women: causative gene or population stratification?" Cancer Research, August 2013

Ellsworth RE, Valente AL, Kane JL, Ellsworth DE, Shriver CD. "Molecular response of the axillary lymph node microenvironment to metastatic colonization" Cancer Research, August 2013

Milburn M, Rosman M, Mylander C, Tafra L. "Is oncotype DX recurrence score (RS) of prognositic value once HER2-positive and low-ER expression patients are removed?" Breast Journal, July-August 2013

Zhou J, Enewold L, Zahm SH, Jatoi I, Shriver C, Anderson WF, Jeffery DD, Andaya A, Potter JF, McGlynn KA, Zhu K. "Breast conserving surgery versus mastectomy: the influence of comorbidities on choice of surgical operation in the Department of Defense health care system." Am J Surg. 2013 Sep; 206(3):393-9. doi: 10.1016/j.amjsurg.2013.01.034.

Yang N, Liu C, Peck AR, Girondo MA, Yanac AF, Tran TH, Utama FE, Tanaka T, Freydin B, Chervoneva I, Hyslop T, Kovatich AJ, Hooke JA, Shriver CD, Rui H. "Prolactin-Stat5 signaling in breast cancer is potently disrupted by acidosis within the tumor microenvironment" Breast Cancer Research, 3 September 2013

Enewold L, McGlynn KA, Zahm SH, Jatoi I, Anderson WF, Gill AA, Shriver CD, Zhu K. "Surveillance mammography among female Department of Defense beneficiaries: a study by race and ethnicity." Cancer. 2013 Oct 1; 119(19): 3531-8. doi: 10.1002/cncr.28242.

Valente AL, Shriver CD, Ellsworth RE. "Gene expression profiling of breast tumors from African American and Caucasian women: are molecular differences meaningful?" AACR Advances in Breast Cancer Research Conference, 3-6 October 2013, San Diego, CA

Field L, Deyarmin B, van Laar R, Shriver CD, Ellsworth RE. "Identification of gene expression profiles associated with different types of breast adipose and their relationship to tumorigenesis" AACR Advances in Breast Cancer Research Conference, 3-6 October 2013, San Diego, CA

Brown S, Shriver CD, Ellsworth RE. "Role of rs9620497 with CRYBB2 expression levels in African American women with invasive breast cancer" ASHG, 22-26 October 2013, Boston, MA

Maskery S, Bekhash A, Kvecher L, Correll M, Hooke JA, Kovatich AJ, Shriver CD, Mural RJ. "Aggregated biomedical information browser (ABB): a graphical user interface for clinicians and scientists to access a clinical data warehouse" AIMA Annual Symposium, 16-20 November 2013, Washington DC

Kovatich AJ, Chen Y, Fantacone-Campbell JL, Wareham JA, Tafra L, Kvecher L, Hyslop T, Hooke JA, Rui H, Shriver CD, Mural RJ, Hu H. "Assays on core biopsies and surgically

resected tumors may result in different subtyping of the invasive breast cancer from the same patient" SABCS, 10-14 December 2013, San Antonio, TX

Chen Y, Kovatich AJ, Fantacone-Campbell JL, Hooke JA, Kvecher L, Kovatich AW, Gallagher CM, Mural RJ, Shriver CD, Rui H, Hu H. "Her2-positive and Her2-negative luminal B subtypes have similar overall survival and histologic grade distributions" SABCS, 10-14 December 2013, San Antonio, TX

Li R, Campos J, Chen Y, Kvecher L, Gdula D, Hoadly K, Shriver CD, Mural RJ, Hu H. "Highly variably expressed exons (HVEE) of cancer genes and survival disparity in human breast cancer" SABCS, 11-14 December 2013, San Antonio, TX

Kovatich AJ, Chen Y, Fantacone-Campbell JK, Wareham JA, Tafra L, Kvecher L, Hyslop t, Hooke JA, Shriver CD, Mural RJ, Hu H. "Cell proliferation marker Ki67 is expressed more in core biopsies than surgically resurrected tumors from invasive breast cancer patients" SABCS, 10-14 December 2013, San Antonio, TX

Field LA, Melley J, Mamula K, Means M, Shriver CD, Ellsworth RE. "Evaluation of epidemiological and molecular differences in African American and Caucasian women with triple negative breast cancer" SABCS, 11-14 December 2013, San Antonio, TX

Ellsworth RE, Valente AL, Blackburn HL, Decewicz A, Deyarmin B, Mamula KA, Shriver CD, Ellsworth DL. "Effect of genomic heterogeneity on breast cancer progression and metastatic spread" SABCS, 11-14 December 2013, San Antonio, TX

Greer LT, Rosman M, Charles Mylander W, Liang W, Buras RR, Chagpar AB, Edwards MJ, Tafra L. "A prediction model for the presence of axillary lymph node involvement in women with invasive breast cancer: a focus on older women" Breast Journal, 30 January 2014

Greenspan R, O'Donnell A, Meyer J, Kane J, Mamula K, Deyarmin B, Larson C, Rigby S, Sturz LA, Lelley J, Shriver CD, Ellsworth RE. "Outcome disparities in African American women with triple negative breast cancer: a comparison of epidemiological and molecular factors between African American and Caucasian women with triple negative breast cancer" BMC Cancer, 4 February 2014

Iida J, Clancy R, Dorchak J, Somiari RI, Somiari S, Cutler ML, Mural RJ, Shriver CD. "DNA aptamers against exon v10 of CD44 inhibit breast cancer cell migration." PLoS One. 2014 Feb 19;9(2):e88712. doi: 10.1371/journal.pone.0088712.

Sturtz LA, Melley J, Mamula K, Shriver CD, Ellsworth RE. "Outcome disparities in African American women with triple negative breast cancer: a comparison of epidemiological and molecular factors between African American and Caucasian women with triple negative breast cancer." BMC Cancer. 2014 Feb 4;14:62.

Maskery S, Bekhash A, Kvecher L, Correll M, Hooke JA, Kovatich AJ, Shriver CD, Mural RJ, and Hu H. "Aggregated biomedical-information browser (ABB): a graphical user interface for

clinicians and scientists to access a clinical data warehouse" Journal of Computer Science & Systems Biology, February 2014

Rummel S, Penatzer CE, Shriver CD, Ellsworth RE. "PSPHL and breast cancer in African American women: causative gene or population stratification?" BMC Genet. 2014 Mar 20;15:38. doi: 10.1186/1471-2156-15-38.

Gallagher C, More K, Hu H, Sicignano N, Masaquel A, Kamath T, Brammer M, Shriver CD, Goehring E, Kapasi A, Jones J. "Discordance rate between two tests used to diagnose patients with HER2-overexpressing breast cancer." AMCP Conference, 1-4 April 2014, Tampa, FL.

Valente AL, Shriver CD, Ellsworth RE. "Gene expression profiling of breast tumors from African American and Caucasian women: are molecular differences meaningful?" American Association for Cancer Research Annual Meeting, April 2014.

Sturtz LA, Deyarmin B, van Laar R, Yarina W, Shriver CD, Ellsworth RE. "Gene expression differences in adipose tissue associated with breast tumorigenesis." Adipocyte. 2014 Apr 1;3(2):107-14. doi: 10.4161/adip.28250.

Valente AL, Kane JL, Ellsworth DL, Shriver CD, Ellsworth RE. "Molecular response of the axillary lymph node microenvironment to metastatic colonization." Clin Exp Metastasis. 2014 Jun;31(5):565-72. doi: 10.1007/s10585-014-9650-9.

Enewold LR, McGlynn KA, Zahm SH, Poudrier J, Anderson WF, Shriver CD, Zhu K. "Breast reconstruction after mastectomy among Department of Defense beneficiaries by race." Cancer. 2014 Jun 25. Doi: 10.1002/cncr.28806

Valente AL, Rummel S, Shriver CD, Ellsworth RE. "Sequence-based detection of mutations in cadherin 1 to determine the prevalence of germline mutations in patients with invasive lobular carcinoma of the breast." Hered Cancer Clin Pract. 2014 Jul 19; 12(1):17. Doi:10.1186/1897-4287-12-17.

Schinkel JK, Zahm SH, Jatoi I, McGlynn KA, Gallagher C, Schairer C, Shriver CD, Zhu K. "Racial/ethnic differences in breast cancer survival by inflammatory status and hormonal receptor status: an analysis of the Surveillance, Epidemiology and End Results data." Cancer Causes Control. 2014 Aug; 25(8):959-68. Doi: 10.1007/s10552-014-0395-1.

V. Conclusions

The annual goal of identify and counseling 100 patients annually at high risk for development of breast cancer while employing risk reduction strategies was achieved

BCTR successfully accrued over 500 patients to the "core" BC-COE protocols through consenting patients in the main BC-COE clinical sites.

BCTR successfully acquired through consented protocol acquisitions over 5,000 specimens on patients with all types of breast diseases and cancer.

As of 23 August 2014 BCTR had banked more than 58K biospecimens in the BC-COE Biorepository, which are then used as the basis for intramural and extramural collaborations for secondary usage research.

During the year BCTR perform focused research on the biospecimens and clinical data collected under the BC-COE Core protocols including global expression analysis of the DNA, RNA, and Protein features and including targeted research into genomic analysis of Stages I, II, and III breast cancer, DCIS, LCIS, and pre-malignant neoplasia, which resulted in CBCP staff presenting findings at peer-reviewed national meetings and publications.

The BCTR at Walter Reed National Military Medical Center held its one day offsite meeting on 18 July 2014 at the Uniformed Services University of the Health Sciences. There were multiple presentations at the offsite, which covered all areas of the CBCP, see attached Agenda.

The John P. Murtha Cancer Center hosted its Second Annual Cancer Research Seminar on Monday 23 June 2013 from 8am-4pm at WRNMMC. There was a presentation given by one of the CBCP scientist on "Characterization of drug targets in triple negative breast cancer tissues".

VI. APPENDICIES

- ATTACHMENT 1: List of personnel receiving pay from the research effort from 24 August 2013 – 23 August 2014.

Current Staff, role and percent of effort on project:

Last, First	Role on Project	Role on Project
Shriver, Craig D.	Principal Investigator	5%
Basham,Janice B	Licensed Practical Nurse	63%
Boone,Jaime J.	Senior Program Manager	19%
Bronfman,Eileen T	Administrative Director	44%
Campbell,Jamie Leigh	Pathologist Assist./Site Coord	44%
Davis, Herma Elaine	Senior Data Manager	17%
Ellsworth,Rachel E.	Cancer Geneticist	48%
Hilton,Karrie R.	Assistant Head Nurse	67%
Holden,Allan	Sr.Data Management Specialist	60%
Hooke,Jeffrey A	Head of Pathology	34%
Joseph,Julie	Research Assistant II	80%
Kovatich,Albert	Scientist	20%
Miskovsky,Vicki Jones	Admin Reviewer CCC Protocols	20%
Patterson,Carol M	Medical Assistant	60%

Pereira,Dianne	Office Manager/Admin. Assist.	53%
Rigatti,Michael Kevin	Research Assistant	39%
Sakura,Sara Denman	Research Protocol Coordinator	51%
Smith,Stephanie R	Research Nurse	66%
Vilakazi,Patricia N.	Biomedical Informatics Coord.	63%
Wareham,Janet Andrea Yoder	Pathologists Assistant	44%
Williamson,Eric	Breast Center Administrator	70%
Zhu,Kangmin	Assoc Dir for Epidemiology	14%
Zingmark,Rebecca N.	Histotechnologist	52%
Cordes,Rosemarie	Research Nurse	8%
Weiss,Raymond B	Physician	38%

- ATTACHMENT 2: Expenditures from 24 August 2013 – 23 August 2014.

Total Cumulative Expenditure
for award W81XWH-12-2-0050
24 August 2013 – 23 August 2014

Personnel:	1,296,382
Consultants:	0.00
Equipment:	13,913
Supplies:	34,977
Domestic Travel:	25,932
Foreign Travel:	1,729
Rent:	21,839
Other Direct Cost:	553,545
Sub award:	3,646,472
Total Direct Cost:	5,594,789
Indirect Cost:	318,753
Fee:	-
Total Program Cost:	**$ 5,913,542**

- ATTACHMENT 3 : Agenda rfom the BCTR one day offsite meeting on 18 July 2014 at the Uniformed Services University of the Health Sciences

Walter Reed National Military Medical Center

Craig D. Shriver, MD, FACS, COL
Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD 20889
craig.d.shriver@health.mil

John P. Murtha Cancer Center Walter Reed Bethesda

CBCP Offsite Meeting

Friday, July 18, 2014

7:30 – 8:00AM	**Registration and Continental Breakfast**	
8:00 – 8:05AM	**Welcome and Announcements**	**Lee Bronfman** **Admin Director, CBCP**
8:05 – 8:20AM	**Opening Remarks**	**Craig D. Shriver** **Director, MCC**
8:20 – 8:40AM	**Greetings from WRI**	**Tom Kurtz** **President/CEO, WRI**
8:40 – 9:00AM	**Translational Breast Research**	**Rachel Ellsworth, PhD**
9:00 – 9:20AM	**The Tissue Bank**	**Stella Somiari, PhD**
9:20 – 9:50AM	**Biomedical Informatics**	**Hai Hu, PhD**
9:50 – 10:05AM		**Break**
10:05 – 10:25AM	**Pathology Update**	**Al Kovatich, Scientist**
10:25 – 10:45AM	**Genetics Update**	**Raymond Weiss, MD**
10:45 – 11:05AM	**Data Team**	**Pat Vilakazi and team**
11:05 – 11:30AM	**Nurse Navigators**	**K.Hilton, S.Smith**

11:30 – 11:50AM	**JMBCC/AAMC**	**P.Felton, J.Joseph, J.Wareham**
11:50 – 12:00PM	**Discussion Wrap Up of Morning**	**Craig D. Shriver**
12:00 – 1:00PM	**Lunch on Your Own**	**USUHS Cafeteria**
1:00 – 1:30PM	**Discussion**	**Craig D. Shriver**
1:30 – 3:30PM	**Visioning for Future**	**Group**
3:30 – 4:00PM	**Concluding Remarks**	**Craig D. Shriver**